TRANSACTIONS

OF THE

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.

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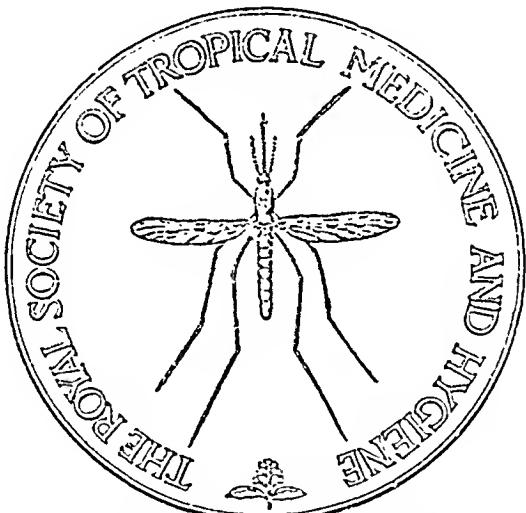
ZONAE TORRIDAE TUTAMEN

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No. 5. *Issued 4th March, 1937.*

Proceedings of a **Laboratory Meeting** held at the Royal Army Medical College, Millbank, London, S.W., at 8.15 p.m., Thursday, 19th March, 1936. Sir ARTHUR BAGSHAWE, *C.M.G.*, M.B., D.P.H., *President*, in the Chair.

DEMONSTRATIONS.

Hygiene Department, Royal Army Medical College.
The present-day feeding of the soldier.

The exhibit was divided into two sections : (A.) **Peace** and (B.) **War**.

(A.) **Peace.**—This section illustrated the feeding of the soldier in England to-day by diagrams, models and actual articles. These included : (1) the actual items of the soldier's diet ; (2) coloured charts showing the distribution of the proximate principles and calories in each item and in the total ration ; (3) a model of the latest cookhouse and dining hall showing how the man's food is prepared, cooked and served.

(B.) **War.**—This section dealt in a similar manner with the feeding of the soldier on active service and included (1) actual items of the active service ration ; (2) a chart showing proximate principles and calorie distributions ; (3) models of a camp cookhouse, travelling kitchens and dining tents, illustrating how the man's food is supplied, prepared, cooked and served in the field ; (4) the

Dr. A. F. Cole.
A case of kitten-bite fever.

Spirillum minus demonstrated in blood of guineapig inoculated with emulsion of excised gland from patient scratched by a sick kitten in Devonshire (England), on 24th August, 1935.

2nd Sept.—After period of no symptoms, developed local tenderness and lymphangitis, with local and general gland enlargement. Twice incised without result.

Treated 3 months in hospital in London : boric foments, saline arm baths, magnesium sulphate compresses, antiphlogistine, ultra-violet rays, arm splinting and autogenous staphylococcal vaccines. Had a dozen pyrexial attacks with erythematous rashes and gland enlargement.

28th Dec., 1935.—In convalescent home during a mild pyrexial attack ; epitrochlear gland excised, and portion injected into guineapig.

28th Jan., 1936.—*Spirillum minus* demonstrated in guineapig. Neokharsivan 0·3 gramme given to patient intravenously. No further attack of fever, whereas during previous 4 months there had been at least fifteen attacks, each lasting 1 to 3 days.

A feature which distressed the patient (a lady aged 31) was the marked development of coarse hair on forearm, dorsum of hand and all fingers of the hand originally scratched.

[A full description of this case was published in the *British Medical Journal* (1936) 28th March, p. 638.]

Dr. S. C. Dyke.
A case of cerebral cysticercosis in man. Numerous " lime corpuscles " in the scolices. (Shown by Dr. Wenyon.)

The brain from the case showed numerous cysts containing scolices of *Taenia solium*. On section the scolices were found to be infiltrated with ovoid corpuscles 15 to 20μ in length. These were soluble in acids and were stained black by Van Kossa's silver nitrate method. They were evidently the " lime corpuscles " which occur commonly in tape worms.

Dr. N. Hamilton Fairley and Dr. F. P. Mackie.
I. Pathological specimens from cases of the following diseases of the small intestine simulating tropical sprue :

(*a*) Gastro-jejuno-colic fistula.
(*b*) Idiopathic steatorrhoea.
(*c*) Regional ileitis.
(*d*) Mesenteric lymph-adenitis.

These specimens were obtained from patients who had died at the Hospital for Tropical Diseases, London. In each case the clinical picture of tropical sprue was simulated. Wasting was marked, the skin was harsh, dry and of a

brownish-yellow colour, the abdominal muscles were atrophied, the parietes flaccid and atonic, and not infrequently a doughy feeling was imparted to the examining fingers. Abdominal distention, especially centrally in the area occupied by the small intestine, was present. In other words, the patients presented the " sprue abdomen." Decreased physical reserve, hypotension, anaemia, muscular cramps and tetany were among the clinical phenomena observed. Biochemical investigations revealed (1) a high faecal fat associated with adequate splitting, (2) a decreased serum calcium, (3) a flat type of glucose tolerance curve, or one showing a subnormal or delayed maximal rise. While all these findings were not invariably present in each case, they constituted a conspicuous feature of the group as a whole. Defective intestinal absorption constituted the basis of the syndrome.

II. A human vermiform appendix infected with *Bilharzia* ova.

This specimen was derived from a patient who had been treated for vesical schistosomiasis some years previously. At operation the appendix was found thickened and studded with subperitoneal nodules similar to, but somewhat larger than, miliary tubercles. Section showed these to be composed of whirls of fibrous tissue encasing dead and calcified ova.

Dr. Norman Hall.
Blood films of ox from Northern Nigeria showing numerous spores of sarcocysts.

This film was taken from the ear of an ox and stained with Giemsa stain. Numerous spores of sarcocysts were seen lying free in the plasma. It is quite possible that such spores might, by inexperienced observers, be mistaken for haemogregarines.

Dr. C. M. Wenyon.
Blood films of ox and horse contaminated with spores of sarcocystis and globidium.

In making blood films from the heart and skin of animals, cysts of sarcocystis and globidium may be damaged with the liberation of spores, which, appearing in the blood films, may be mistaken for blood parasites.

Dr. C. A. Hoare.
Trypanosoma simiae, the cause of acute porcine trypanosomiasis in Africa.

A demonstration was given of preparations of trypanosomes from cases of acute trypanosomiasis in the domestic pig, from the Belgian Congo, and of one of the original films of *Trypanosoma simiae* made by Sir DAVID BRUCE. The porcine parasite was found to be identical with *T. simiae*, which—on re-examination—proved to be polymorphic, comprising in addition to the classical *simiae*-form, a *rodhaini*-like form and a *congolense*-like form. Specimens of these

three types were exhibited. A full account of this trypanosome and of its relation to the disease in pigs was published in the last issue of these TRANSACTIONS (Vol. xxix, p. 619).

Mr. B. Jobling.

Morphological differences, in the larval stages, between autogenous and anautogenous races of *Culex pipiens*.

There are two races of *Culex pipiens* in Europe, which differ biologically as follows :—

The females of the anautogenous race feed chiefly on birds and only rarely attack man. They cannot mate in confinement, are hibernating and require a blood meal before oviposition. On the other hand, the females of the autogenous race attack man readily. They are non-hibernating, can mate in confinement and can lay fertile eggs without any meal.

The adults of these two races have different coloration. The autogenous mosquito is slightly lighter in colour than the anautogenous. But the most distinct character is present in the larvae, and by this character they can easily be separated from each other in mixed culture.

The siphon of the larva of the autogenous mosquito is much thicker and shorter than that of the larva of the anautogenous mosquito. The anal gills of the autogenous mosquito are short. In the third and the fourth stage larvae the gills are about half as long as the ventral brush of the anal segment, whereas in the larvae of the anautogenous mosquito the gills are about as long as, or a little longer than, the ventral brush. There is also a difference in the structure of the ventral brush, of which the tufted hairs have many more branches in the autogenous than in the anautogenous mosquito. This is especially distinct in the last two stages of the larvae.

Dr. P. Manson-Bahr.

Demonstration of paintings, drawings and slides.

Dr. MANSON-BAHR demonstrated a series of paintings which he had made of the sigmoidoscopic appearances of pathological conditions of the colon associated with dysenteric symptoms. Slides and paintings of exudates containing ova and protozoa, together with drawings of microscopical sections of dysenteric conditions were also exhibited.

Major-General Sir John Megaw.

A simple method of checking the strength of stock mixtures of quinine.

This is a slight modification of the methods described in the *Indian Medical Gazette* of May, 1928, p. 244 ; and July, 1929, p. 378. The requirements are :—

(1) Quinine test reagent consisting of pure phosphotungstic acid 1 oz., dilute sulphuric acid (B.P.) 5 oz., rectified spirit 12 oz.

(2) Control solution of quinine of the same strength and composition as the mixture to be tested, *e.g.*, 10 grains to the ounce.

(3) A few test tubes of equal calibre—about ⅛ inch by 3 inches.

(4) A marked pipette to deliver about 2 c.c.

(5) A small pipette marked so as to measure about 0·2 c.c.

Method.

Put 2 c.c. of the test reagent in each of two test tubes. Add 0·2 c.c. of the control solution to one tube and mix well immediately by reversing the tube five or six times while keeping it closed. Add 0·2 c.c. of the mixture under test to the other tube, mix well immediately. Then mix the contents of both tubes simultaneously and place the tubes in a vertical position.

If the two solutions have been treated in exactly the same way any difference in their strength will be indicated within 10 minutes by differences in the height of the supernatant liquid, and in half an hour by differences in the height of the precipitates.

If the stock mixture is stated to be 5 grains to the ounce, the control solution must, of course, be the same and 0·4 c.c. of each of the quinine solutions must be added to the tubes containing the test reagent.

If no measuring pipettes are available a still simpler but less accurate test is to add one drop of the control solution to 1 c.c. of the test reagent and one drop of the mixture under test to 1 c.c. of the reagent in another tube of the same calibre. The contents of each tube must be mixed as described above. Considerable variations in strength will be shown by differences in the opacity of the contents of the tubes. The drops should be added by the same pipette, held at the same angle in each case.

Neither method is suitable for accurate quantitative estimates of the strength of mixtures. Do a few preliminary tests, first using two solutions of the same strength, and then solutions of different, but known, strengths until the technique is mastered. Note that if the quinine solution is not *immediately* mixed, after being added to the reagent, variations in the precipitate will result.

The purity of the stock quinine powder can be tested by comparing a solution made up on the spot with a control solution made from pure quinine.

Dr. D. R. P. Murray.

Factors which affect the spread of oil upon water.

Although nearly all oils spread on clean water, they may fail to spread on dirty water owing to the lowered surface tension ; certain substances are therefore often added to anti-malarial oils to aid the spread. Some of the complications arising from this practice were demonstrated.

While fatty acids or vegetable oils increase very much the spreading power of mineral oils, their own great tendency to form monomolecular films on which

further oil will not spread leads to an instability of the film. If the film once gets broken by wind, the monomolecular layer prevents it from ever rejoining up to a continuous film. Some distilled cuts of petroleum contain naphthenic acid to such a concentration that this phenomenon occurs without adding any other substance.

In the " bottoms " or undistilled fraction of petroleum, however, there occurs a substance (or substances) which aids spread over dirty surfaces without imparting instability, and which in addition counteracts the effect of fatty acid or naphthenic acid already present. Such a fraction, therefore, forms a most valuable constituent for a stable-spreading oil.

On the other hand, before deciding on the desirability of such incorporations into an anti-malarial oil, attention has to be given to the fact that some films, though stable, become useless or worse than useless through a " toughening " which prevents the oil from penetrating the tracheal system of the mosquito larvae. The particular compromise to be made must be determined by local conditions, such as whether the water is flowing or stagnant, its accessibility, ease of reapplication of oil, etc.

Professor F. W. O'Connor.

I. Tracings of the movements of sheathed embryos of *Wuchereria bancrofti* in heparinized blood on microscope slides at room temperature, St. Croix, Virgin Islands, U.S.A., 13th January, 1936.

II. Microfilariae in the blood of the ground dove of St. Croix, Virgin Islands.

(Shown by Col. Clayton Lane.)

I. The tracings were those of six microfilariae and are made up largely of a series of intersecting loops. The respective distances covered by the larvae were 3·588 mm. in 15 minutes (0·239 mm. a minute), 2·028 mm. in 10 minutes (0·203 mm. a minute) when there was no cover glass ; 5·148 mm. in 20 minutes (0·258 a minute) and 9·516 mm. in 25 minutes (0·381 mm. a minute) when a cover was present ; and 3·354 mm. in 7 minutes (0·479 mm. a minute) and 4·134 mm. in 16 minutes (0·259 mm. a minute) when the specimen was covered and ringed with vaselin. The average rate of travel was 0·363 mm. a minute.

II. The specimens showed an extraordinarily heavy infection with round-tailed, sheathed microfilariae in the blood of the jugular vein of the ground dove, *Chaemepelia passerina trochila* (Bonaparte) shot on St. Croix at 11.30 a.m.

Professor F. W. O'Connor and Mr. Harry Beatty.

The early migrations of *Wuchereria bancrofti* in *Culex fatigans*.

(Shown by Col. Clayton Lane.)

These observations are fully described in the Communication on p. 125.

Lieut.-Colonel H. E. Shortt.

I. Canine piroplasmosis.

(1) Development of *Babesia canis* in the dog tick (*Rhipicephalus sanguineus*). Pre-club forms, showing cytoplasmic clefts, in a phagocytic cell of the body cavity simulating a cyst.

(2) Infective stages (sporozoites) of *Babesia canis* in the acini of the salivary glands of *Rhipicephalus sanguineus*.

II. Kala-azar.

Leishmania donovani in the nasal secretion of a case of Indian kala-azar.

III. Rabies.

Negri bodies in a section of hippocampus major of the dog, stained to show internal structure. Iron haematoxylin, following fixation in Flemming's osmic acid fixative.

Professor J. Gordon Thomson.

The occurrence of *Glugea anomala* (Moniez, 1887, Gurley, 1893) in the Stickleback *Gasterosteus aculeatus* in ponds and streams in England.

Sticklebacks heavily infected with this microsporidian were captured in ponds in North London. The genus *Glugea* was created by THÉLOHAN (1891) for the parasite which caused conspicuous cysts in the subcutaneous tissues of sticklebacks. These cysts, the so-called " Glugea cysts," grow to a size of 3 to 4 mm. in diameter and are therefore quite visible to the naked eye. The infections vary in intensity from one or two cysts to many hundreds scattered over various parts of the body. Many of the animals showed infections of both eyes resulting in blindness. Death ensues owing to the fact that interference with the movement and sight of the fish prevents feeding. Large " Glugea cysts " frequently occur in the branchial cavities.

Sections through a " Glugea cyst " when stained with Weigert's iron haematoxylin and Van Gieson's stain show that the outer wall is composed of concentric layers of fibrous tissue cells which are a tissue reaction of the host. Inside this is a homogeneous wall which stains pink with Van Gieson's stain and shows no cellular structure. This latter part of the cyst wall is evidently formed by the parasite itself and is not a tissue reaction. Inside the cyst wall is the protoplasm of the hypertrophied host cell and the growing parasite which shows three distinct zones :—

(*a*) Immediately under the homogeneous wall of the cyst a continuous protoplasmic area of variable thickness is seen.

(*b*) In the zone next to this there are numerous nuclei of two kinds. The large nuclei, which are the nuclei of the original hypertrophied host cell, are elongated and, amongst these, smaller nuclei of the parasite itself are found.

At the inner part of this zone numerous vacuoles are seen containing uninucleated small cells called vacuole cells (sporonts).

(c) The central portion of the cyst is filled with minute oval spores about 3.5μ in length. When these spores are treated with various strengths of hydrogen peroxide a single long polar filament about 150μ in length is extruded with explosive violence, a process which can be beautifully observed by dark-ground illumination.

Sticklebacks are infected by the ingestion of these mature spores. A clean stickleback was given experimentally a large " Glugea cyst " which it greedily swallowed. Thirteen days later a small cyst could be detected in the cornea of each eye and with the aid of a low power lens several small subcutaneous nodules could be seen under the skin. On the 29th day after the infective feed the fish died and by the aid of a lens a massive infection of the subcutaneous tissues all over the body could be detected accompanied by an infection of both eyes.

The geographical distribution of this microsporidian in sticklebacks is given by KUDO (1924) as France, England, Russia, Germany and Belgium. WOODCOCK (1904) reported its presence in England.

REFERENCES.

KUDO, R. (1924). A biologic and taxonomic study of the Microsporidia. *Illinois biol. Monogr.*, ix, Nos. 2 & 3.
WOODCOCK, H. M. (1904). On Myxosporidia in flat-fish. *Proc. & Trans. Lpool. biol. Soc.*, xviii.

ngs of an **Ordinary Meeting** of the Society, held at
House, 26, Portland Place, London, W.1, at 8.15 p.m.,
on Thursday, 21st May, 1936.
Arthur Bagshawe, *C.M.G.*, M.B., D.P.H., *President*,
in the Chair.

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The President announced that His Majesty the King
had graciously consented to become Patron of the Royal
Society of Tropical Medicine and Hygiene in succession to
His late Majesty King George V.

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PAPERS.

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TROPICAL SPRUE WITH SPECIAL REFERENCE TO INTESTINAL ABSORPTION.

Part I.

BY

N. HAMILTON FAIRLEY, M.D., D.Sc., F.R.C.P.

Assistant Physician and Director of Pathology, Hospital for Tropical Diseases, London.

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Part I.

The present paper summarises certain observations and reflections which are the outcome of a personal study of over 450 cases of tropical sprue, first in India prior to the discovery of liver extract and subsequently in London amongst patients who have been invalided back with this disease.*

The difficulty of presenting the subject has been considerably increased by the tendency in recent years, especially in America and on the Continent, to identify coeliac disease of childhood with idiopathic steatorrhoea of adult life and tropical sprue. While the absence of intestinal lesions at autopsy and deranged intestinal absorption are common to all three diseases, the inclusion of tropical sprue is, in the writer's opinion, neither justifiable nor advisable in the present state of knowledge. Idiopathic steatorrhoea itself includes two groups of cases, either of which may be associated with mega-colon : (1) adult coeliac disease, cases of which present bony deformity and give a history of prolonged intestinal disturbance in childhood ; (2) so-called non-tropical sprue occurring in adults who have never lived in the tropics. Both are much more severe diseases than tropical sprue and frequently terminate fatally despite modern treatment. Tropical sprue, on the other hand, is exceedingly amenable to correct treatment and in the absence of complications invariably justifies a good prognosis. Unless specifically stated the subsequent remarks concern only tropical sprue.

Owing to the megalocytic nature of the anaemia common to sprue and pernicious anaemia some divergence of opinion still exists in regard to the duality of the two diseases. For this reason throughout this paper attention has been directed from time to time to certain essential differences in their pathology, symptomatology, biochemistry and response to treatment.

I.—PATHOLOGY OF TROPICAL SPRUE.

Necropsy reveals a dry, grayish or brownish-yellow skin, marked emaciation, scanty subcutaneous tissue and atrophied, pale muscles. Similar atrophy of the muscles of the tongue associated with smoothness and thinning of the mucous membrane with atrophy of the fungiform and filiform papillae is not uncommon.

(a) The Gastro-Intestinal Tract.

The published and unpublished studies by MACKIE and FAIRLEY (1934) on postmortem material, examined within a few minutes to 2 hours after death, are in general agreement with those of THAYSEN (1931) and indicate that sprue may run its full course over a period of many years and come to autopsy without

manifesting any of the specific degenerative or inflammatory changes in the gastro-intestinal tract which throw light on its pathogeny. Undoubtedly many of the changes previously described as occurring in the alimentary tract in tropical sprue have been due to early necrobiotic change, autodigestion and post-mortem distension of the gut. Our recent microscopic examination of freshly fixed material has shown intact oxyntic cells and normal pyloric and Brünner's glands. The "withering of the villi," originally described by MACKIE and FAIRLEY (1929) from material collected in the tropics 8 hours or longer after death, has been conspicuously absent in tissues more recently obtained in London at a time when peristaltic movement in the intestine was still visible. Similarly, ulceration of the mucosa was absent. Occasionally isolated ulcers of the small intestine have been recorded in tropical sprue, but they are not to be regarded as the essential pathological lesion as suggested by MANSON-BAHR (1924), but rather as a complication only occasionally encountered. Thinning of the gut associated with disappearance of fat deposits may occur, but this is probably only part of the general wasting characteristic of the disease and not a specific atrophy.

(b) Visceral Changes.

In a series of ten cases which we personally have autopsied the average percentage decrease in weight was 27·2 for the liver, 20·5 for the kidneys, 34·1 for the heart and 35 for the spleen. The Prussian blue reaction may be positive in the liver, spleen and kidneys.

In sprue the heart is often small, in this regard resembling that seen in Addison's disease of the adrenal glands. Microscopic examination reveals brown atrophy. In both these respects the findings differ from those of Addisonian pernicious anaemia, where the heart is not reduced in size and where fatty degeneration is commonly observed. Furthermore, in Addisonian anaemia, the body is well nourished, the subcutaneous fat abundant and bright lemon-yellow in colour, the muscles red and the spleen enlarged.

(c) Bone Marrow.

The bone marrow after death has been specially studied by FAIRLEY, MACKIE and their associates (1926), and MACKIE and FAIRLEY (1929). Their findings indicated that true megaloblastic hyperplasia of the red marrow occurs in this disease. In some instances the whole of the long bones was filled with megaloblastic marrow, in others megaloblastic hyperplasia was almost or entirely restricted to the short bones and the ribs. A feature of special interest was that the yellow or greenish-yellow fatty marrow in the long bones frequently presents a peculiar gelatinous appearance similar to that seen in certain nutritional diseases.

KRJUKOFF (1928) investigated the rib marrow obtained by biopsy and found megaloblastic changes in each of his sixteen cases. RHOADS and CASTLE (1933) examined sternal marrow in twenty-two cases of tropical sprue with macrocytic

anaemia ; in every instance there was proliferation of megaloblasts. Recently, in conjunction with MACKIE, I have been studying marrow smears obtained by sternal puncture during life. Primitive cells of the red cell series of all types are encountered. By no means all of them conform to the classical megaloblast as described by EHRLICH, for their cytoplasm is frequently neither polychromasic nor orthochromic ; they do, however, conform to the megaloblast as described by PEABODY (1927) in his important paper on the bone marrow in Addisonian anaemia. Many of these cells are as large as myelocytes, possess a large pale-stained nucleus with a fine reticular and nodal structure, multiple nucleoli and a non-granular basic cytoplasm. By TURNBULL (1934) they would be classified as primitive haemocytoblasts.

While megaloblastic hyperplasia is explicable in terms of a deficiency of haemopoietic principle or P.A. factor reaching the bone marrow, the peculiar gelatinous appearance of the yellow marrow in the long bones calls for explanation. Like the visceral atrophy it probably has a nutritional origin dependent on an inability of the small intestine to absorb certain food constituents as indicated by biochemical studies. The nutritional defect, however, is not one of pure starvation since protein is still satisfactorily absorbed and metabolised in the body. This is probably the reason why the degree of visceral atrophy is not identical with that observed in starvation. VOIGT showed in starving animals that 97 per cent. of the adipose tissue may have disappeared and the store of glycogen in the liver may be so depleted as to have diminished that organ by one-half, yet the brain and heart had lost only 3 per cent. of their original weight.

II.—CLINICAL FEATURES.

The onset may be either insidious or sudden with (1) diarrhoea, (2) sore tongue and apthous ulcers in the mouth, (3) abdominal discomfort, meteorism and flatulence. In acute cases fever may be present for the first two or three days, but in the absence of complications the course of the disease is afebrile. Generally the temperature is subnormal.

(a) Typical or Gastro-Intestinal Sprue.

In its typical and well-established form tropical sprue is characterised by an afebrile diarrhoea which may commence early in the morning and recur throughout the day, though the looseness of the bowels often stops before mid-day. The stools are variable in number, of a loose, bulky, fatty consistency, pale or occasionally even white in colour and commonly gaseous. The call to defaecation is often urgent and evacuation of the bowel may occur with explosive violence. Occasionally during acute exacerbations an enteritic type of diarrhoea with frequent brown fluid stools may temporarily supervene. Intestinal flatulence and attacks of abdominal distension are outstanding features, especially after the ingestion of carbohydrate foods. There is considerable loss of weight, marked asthenia and lowering of blood pressure. Not infrequently sore tongue and apthous ulcers develop, the pain may be agonizing and is increased by taking

hot fluids, acids, condiments, and the like. Dysphagia, retrosternal oesophageal pain and vomiting are occasionally present.

The skin is harsh and dry and sometimes of a parchment-like consistency. It often presents a dirty, brownish-yellow discoloration quite distinct from the lemon-yellow tinting of the skin seen in pernicious anaemia. Brown pigmentation may be present. The nails are brittle and often ridged. The tongue is clean, and red in colour unless the anaemia be advanced. From time to time patches of inflammation develop involving its sides and tip and crops of apthous ulcers may implicate the cheeks, floor of the mouth and tongue. As the process advances the dorsum of the tongue becomes smooth and glossy from atrophy of the filiform and fungiform papillae.

The abdomen is distended and its walls remarkably thinned owing to disappearance of subcutaneous tissue and atrophy of muscle. Visible peristalsis is commonly observed. The knee jerks are frequently sluggish or absent, paraesthesia may be complained of and patches of anaesthesia occasionally demonstrable. Sometimes there is difficulty in performing complicated muscular movements like knitting, but these features are in my experience invariably due to neuritis. Ataxia, increased knee jerks, a Babinski response, absent superficial abdominal reflexes, and sphincter complications or trophic ulcers, characteristic of subacute combined degeneration of the spinal cord so common in pernicious anaemia and occasionally recorded in idiopathic steatorrhoea have never been encountered in a personally observed series of over 450 cases of sprue. No mention of this complication was made by Low (1928) or MANSON-BAHR (1930) in their clinical analysis of cases, nor by ASHFORD (1932) in a review of 3,000 cases at Porto Rico. In the only case of sprue of the present series coming to autopsy in which cord involvement was thought to be even remotely possible, the brain and spinal cord were examined by Dr. J. G. GREENFIELD. No abnormality other than some chromolytic changes in the anterior horn cells was noted. Myelin degeneration was definitely absent.

Pains in the muscles, and tetany may occasionally prove troublesome. Apart from carpo-pedal spasm tetany may be associated with sufficient muscular stiffness and cramp to make walking difficult. Trousseau's and Chvostek's signs are usually elicited when the serum calcium falls below 7·0 mg. per 100 c.c.

Occasionally in severe chronic cases oedema of the feet and legs may develop and another uncommon feature is purpuric skin eruptions. Both are much rarer in tropical sprue than in idiopathic steatorrhoea.

(b) Atypical Types.

Occasionally patients feel " out of sorts " and develop sore tongue and apthous ulcers of the mouth a few weeks before sprue diarrhoea commences, or between relapses at a time when they are otherwise fairly well (lingual sprue). Even more rarely in the winter a patient who has had sprue may develop a respiratory infection and relapse with palpitation, dyspnoea on exertion,

anaemia ; in every instance there was proliferation of megaloblasts. Recently, in conjunction with MACKIE, I have been studying marrow smears obtained by sternal puncture during life. Primitive cells of the red cell series of all types are encountered. By no means all of them conform to the classical megaloblast as described by EHRLICH, for their cytoplasm is frequently neither polychromasic nor orthochromic ; they do, however, conform to the megaloblast as described by PEABODY (1927) in his important paper on the bone marrow in Addisonian anaemia. Many of these cells are as large as myelocytes, possess a large pale-stained nucleus with a fine reticular and nodal structure, multiple nucleoli and a non-granular basic cytoplasm. By TURNBULL (1934) they would be classified as primitive haemocytoblasts.

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hot fluids, acids, condiments, and the like. Dysphagia, retrosternal oesophageal pain and vomiting are occasionally present.

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The abdomen is distended and its walls remarkably thinned owing to disappearance of subcutaneous tissue and atrophy of muscle. Visible peristalsis is commonly observed. The knee jerks are frequently sluggish or absent, paraesthesia may be complained of and patches of anaesthesia occasionally demonstrable. Sometimes there is difficulty in performing complicated muscular movements like knitting, but these features are in my experience invariably due to neuritis. Ataxia, increased knee jerks, a Babinski response, absent superficial abdominal reflexes, and sphincter complications or trophic ulcers, characteristic of subacute combined degeneration of the spinal cord so common in pernicious anaemia and occasionally recorded in idiopathic steatorrhoea have never been encountered in a personally observed series of over 450 cases of sprue. No mention of this complication was made by LOW (1928) or MANSON-BAHR (1930) in their clinical analysis of cases, nor by ASHFORD (1932) in a review of 3,000 cases at Porto Rico. In the only case of sprue of the present series coming to autopsy in which cord involvement was thought to be even remotely possible, the brain and spinal cord were examined by Dr. J. G. GREENFIELD. No abnormality other than some chromolytic changes in the anterior horn cells was noted. Myelin degeneration was definitely absent.

Pains in the muscles, and tetany may occasionally prove troublesome. Apart from carpo-pedal spasm tetany may be associated with sufficient muscular stiffness and cramp to make walking difficult. Trousseau's and Chvostek's signs are usually elicited when the serum calcium falls below 7·0 mg. per 100 c.c.

Occasionally in severe chronic cases oedema of the feet and legs may develop and another uncommon feature is purpuric skin eruptions. Both are much rarer in tropical sprue than in idiopathic steatorrhoea.

(b) Atypical Types.

Occasionally patients feel " out of sorts " and develop sore tongue and apthous ulcers of the mouth a few weeks before sprue diarrhoea commences, or between relapses at a time when they are otherwise fairly well (lingual sprue). Even more rarely in the winter a patient who has had sprue may develop a respiratory infection and relapse with palpitation, dyspnoea on exertion,

dyspepsia and severe megalocytic anaemia in the absence of intestinal symptoms (gastric sprue), and if appropriate treatment be withheld diarrhoea soon supervenes. In all such cases complete biochemical as well as haematological investigations should be undertaken. Were this done, early corroborative evidence of intestinal involvement would more often be forthcoming and confusion with pernicious anaemia avoided.

In other cases the classical intestinal features of sprue may be present without megalocytic anaemia (intestinal sprue) but sooner or later in the course of the disease anaemia of this type supervenes. In most instances there is an inability to utilise adequately both fat and carbohydrate foodstuffs and the complete abdominal sprue syndrome develops. Occasionally, however, one or other is predominantly affected. If fat be specially implicated there is steatorrhoea with or without tetany ; if carbohydrate is poorly tolerated intestinal flatulence, abdominal distension and acid gaseous stools are found. That the condition is not dependent on faulty digestion of starch is shown by the absence of an excess of undigested starch granules in the faeces.

III.—BIOCHEMICAL AND OTHER DATA RELATING TO DEFECTIVE ABSORPTION OF FOOD CONSTITUENTS.

In this section biochemical and other data bearing on the question of the absorption of fundamental food elements will receive consideration.

(a) Fat Analysis of the Faeces.

In well-established cases of sprue, over 50 per cent. of the ingested fat may appear in the faeces, whilst in patients on an ordinary mixed diet as much as 50 grammes of fat may be passed every 24 hours. The estimation of the total fat excreted over several days and its expression as a percentage of the total fat intake during that period is a laborious undertaking and has not proved possible in the majority of patients at the Hospital for Tropical Diseases, most of whom are private cases. Slight reduction in the fatty acid content of the blood (average $= 25 \cdot 9$ mg. per 100 c.c.) was found by FAIRLEY, MACKIE and their colleagues (1926) in Bombay, using Bloor's method in a series of twenty-one cases of tropical sprue. The estimation of blood fat, however, is not entirely satisfactory and in the absence of a suitable fat tolerance test for clinical use no further observations on this aspect of the question have been made.

On the other hand, faecal fat analysis of specimens of dried stools collected while the patient was taking an ordinary mixed diet (with an approximate ratio of protein : fat : carbohydrate $= 1 \cdot 0 : 1 \cdot 0 : 5 \cdot 0$) has been carried out almost as a routine procedure. The technique of HOLT, COURTNEY and FALES (1919) was adopted and the results in the last seventy cases of tropical sprue are epitomised in the accompanying Table 1.

TABLE 1.

FAT ANALYSIS OF DRIED FAECES IN SEVENTY CASES OF TROPICAL SPRUE.
(Method of Holt, Courtney and Fales.)

TOTAL FAT.		SPLIT FAT.	
Total Fat. Per cent.	Number of Cases.	Fatty Acids and Soaps. Per cent.	Number of Cases.
Under 25	14	Under 40	1
25 to 29·9	7	40 to 50	9
30 to 39·9	14	50 to 60	16
40 to 49·9	21	60 to 70	16
50 to 59·9	9	70 to 80	20
Over 60	5	Over 80	8
Total	70	Total	70
Average total fat = 39·1 per cent.		Average split fat = 64·8 per cent.	

From this it will be seen that in fifty-six out of seventy cases, or 80 per cent., a single analysis showed that the total fat exceeded 25 per cent. of the dried faeces. In the remaining fourteen cases it fell within normal limits. Sprue stools are excessively bulky and had the total fat excreted in the 24 hours been estimated and expressed in terms of the percentage of ingested fat it is highly probable that in at least some of these higher values than normal would have been obtained.

Though the split fat averaged 64·8 per cent. and approximated closely to the normal, some variation was found in the extent to which neutral fat was split into fatty acids and soaps. In ten cases, 50 per cent. or less was split, whilst in eight others the split fat exceeded 80 per cent. of the dried faeces (Table 1). From these results it follows that in the majority of cases of tropical sprue fat splitting is normal ; in a few it is defective and in a few excessive.

The beneficial effect of efficient treatment on the fat content of the faeces is strikingly illustrated when fat analysis of the stools is performed on admission and discharge of the patient. In Table 2 the results in a series of twenty cases of tropical sprue are analysed.

It will be seen that on discharge the faecal fat averaged 26·2 per cent. of which 63·1 per cent. was split—values which are very close approximations to the normal. In many other cases examined at a longer interval, after they were

TABLE 2.

THE FAT CONTENT OF THE DRIED FAECES IN SPRUE.

(20 Cases.)

	On Admission.		On Discharge.	
	Total Fat per cent.	Fatty Acids and Soaps per cent. (Split Fat.)	Total Fat per cent.	Fatty Acids and Soaps per cent. (Split Fat.)
Maximum	67·3	97·7	38·5	82·4
Minimum	32·0	38·4	16·4	36·5
Mean	48·2	66·5	26·2	61·3

clinically cured, a normal value for faecal fat has been found (10 to 25 per cent. of the dried faeces).

It can be definitely stated that as far as tropical sprue is concerned cure is associated with restoration of the faecal fat to normal limits when the patient is on a normal balanced diet. If the faecal fat remains abnormally high the patient is not cured.

(b) Serum Calcium and Phosphorus.

The association of tetany with tropical sprue was first noted by CANTLIE (1913). H. H. SCOTT (1923) drew attention to the low ionic calcium values which he obtained using the method of Vines for its determination—a result confirmed by MACKIE, FAIRLEY and their colleagues (1926). ASHFORD and HERNÁNDEZ (1926), BAUMGARTNER and SMITH (1927) and FAIRLEY (1930) later recorded investigations of the total calcium content of the serum. The data here recorded both in regard to calcium and phosphorus determinations in tropical sprue are in entire agreement with the findings published in the last-mentioned paper and will be only briefly discussed.

Serum Calcium.—In the present series the total serum calcium was investigated in 80 cases using the technique of Kramer and Tisdall. The results are recorded in Table 3.

The average value was 8·8 mg. per 100 c.c., the minimum 5·1 mg. and the maximum 12·0 mg. Twelve patients suffered from tetany; in five the serum value varied from 5·1 to 6·0 mg., in six it varied from 6·1 to 7 mg., whilst in the last it equalled 7·2 mg. In forty-two patients normal values of over 9·0 mg. were found.

All the patients with tetany presented Trousseau's and often Chvostek's signs and some complained of difficulty in walking owing to muscular stiffness and cramp. As a rule the response to calcium *per os* was excellent provided the patient simultaneously received a graded high protein diet, low in fat and carbohydrate, and oral liver extract. This is well illustrated in Graph I which

TABLE 3.

THE SERUM CALCIUM IN SPRUE.
(80 Cases.)

Serum Calcium. Mg. per 100 c.c.	Number of Cases.	Cases with Tetany.
5·1 — 6·0	5	5
6·1 — 7·0	6	6
7·1 — 8·0	8	1
8·1 — 9·0	19	Nil.
Over 9·0	42	Normal

The average for the series = 8·8 mg. per 100 c.c.
Minimum = 5·1 mg.; maximum = 12·0 mg. per 100 c.c.

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Inorganic Phosphorus.—The inorganic phosphorus was estimated by Brigg's modification of Bell and Doisey's method in thirty-two typical cases of tropical sprue—a total of sixty observations being recorded. The results may be studied in the accompanying table.

TABLE 4.

THE PLASMA INORGANIC PHOSPHORUS IN THIRTY-TWO CASES OF TROPICAL SPRUE.
(Sixty Observations.)

Inorganic Phosphorus. Mg. per 100 c.c.	Number of Cases.
1·6 – 2·0	3
2·1 – 2·5	4
2·6 – 3·0	18
3·1 – 3·5	13
3·6 – 4·0	13
4·1 – 4·5	6
4·6 – 5·0	3

Average for the series = 3·3 mg. per 100 c.c.
Minimum = 1·6 mg. per 100 c.c. ; maximum = 4·8 mg. per 100 c.c.

Authorities vary in regard to the normal range of inorganic phosphorus in the adult. HARRISON (1930) states it to be 2·0–4·0 mg., PETERS and VAN SLYKE (1931) 2·5–5·0 mg., while LINDER and HARRIS (1930) accept 3·5–4·5 mg. per 100 c.c. in their studies on calcium and phosphorus metabolism in diarrhoea associated with tetany. For purposes of the present investigation 2·5 to 5·0 mg. has been taken as the normal range. In the series under review 26 of the 32 cases fell within these normal limits. The average was 3·3 mg. per 100 c.c., the maximum 4·8 mg. and the minimum 1·6 mg. per 100 c.c. In six cases it was below 2·5 mg. per 100 c.c. The findings indicate that in tropical sprue the inorganic phosphorus falls either within or below normal limits.

Commentary.

In parathyroid tetany blood phosphorus is high, whilst the total calcium value is low, but where there is defective absorption of lime salts in the intestine, low serum calcium associated with a low or normal inorganic phosphorus value is found. These results therefore support the view that the low total serum calcium values are caused by defective absorption in the intestine rather than by derangement of the parathyroid glands. Not all cases of steatorrhoea show hypocalcaemia, but where hypocalcaemia is found the faecal fat is abnormally high and invariably so in the presence of tetany. Furthermore, the difficulty of restoring the serum calcium to normal values while steatorrhoea persists has already been referred to. Several explanations have been suggested regarding the basis of hypocalcaemia in steatorrhoea. The presence of excessive amounts of fat may physically interfere with calcium absorption, whilst the excess of fatty acids by combining with the ingested calcium in the intestine to form insoluble calcium soaps may produce the same result. In addition there may be defective absorption of fat soluble Vitamin D which not only influences calcium absorption, but enables the serum to hold large amounts of calcium and inorganic phosphorus without upsetting the phosphorus and calcium balance. LINDER and HARRIS (1930) concluded that inadequate absorption of Vitamin D was the major cause of tetany in steatorrhoea.

The administration of Vitamin D or ultra-violet irradiation, however, appears to be of less importance in tropical sprue in accelerating restoration of the blood calcium than in either coeliac disease or idiopathic steatorrhoea. Only three cases of tetany in the present series received irradiated ergosterol or ultra-violet irradiation, yet the serum calcium invariably returned to normal limits with calcium *per os* and low fat diet. Unfortunately the same cannot be said for idiopathic steatorrhoea. Recently I treated a patient with this disease who died after the blood had been restored to normal, with uncontrollable diarrhoea and tetany despite intensive treatment. In the carefully investigated case of tropical sprue with steatorrhoea and tetany recorded by LINDER and HARRIS (1930) it is interesting to note that on the 53rd day, after 35 days on irradiated ergosterol (36,000 units daily), a diet high in calcium and phosphorus was given with the result that a positive daily balance of about 600 mg. each of calcium and phosphorus was established. In spite of this, tetany became more frequent and severe and the serum calcium and phosphorus fell again to 7·3 and 2·0 mg. per 100 c.c. respectively. On the 78th day the fat in the diet was reduced to only 15 grammes. The tetany disappeared 2 days later ; the serum calcium and phosphorus soon rose to normal values and the patient's condition improved remarkably.

In contra-distinction to coeliac disease and idiopathic steatorrhoea, hypocalcaemia in tropical sprue never leads to osteomalacia, bony deformity or spontaneous fracture. Furthermore, gross osteoporosis must be relatively rare, for in several selected cases examined in the present series, Dr. MATHER CORDINER

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failed to demonstrate its presence when employing as a control a person of the same sex and age group. Some degree of osteoporosis is to be expected in chronic cases with persistent hypocalcaemia and further investigations on the point are being carried out.

(c) *Glucose Utilisation in Sprue.*

THAYSEN (1926) first called attention to the fact that the blood sugar in idiopathic steatorrhoea may show a remarkably small rise after the ingestion of glucose. A blood sugar curve was regarded as being low if the maximum rise did not exceed 40 mg. per 100 c.c. following the administration of 60 grammes of glucose, provided the blood was examined at intervals of 15 minutes or less. Later THAYSEN and NORGAARD (1929) described low blood sugar curves as being generally characteristic of idiopathic steatorrhoea, under which title they identified coeliac disease, non-tropical and tropical sprue. Four cases of tropical sprue, two of which had recovered clinically, were reported. Normal maximal rises of 79, 67 and 80 mg. per 100 c.c. were recorded in three cases, but in the fourth case a maximal rise of only 21 mg. per 100 c.c. was noted at the first examination ; 14 months later the test was repeated when a rise of 51 mg. per 100 c.c. was recorded.

THAYSEN (1929) held in all three diseases which he identified under the title " idiopathic steatorrhoea or non-tropical sprue," that some disturbance in the internal regulation of blood sugar was responsible and not the destruction of glucose or its retarded or impaired absorption in the intestine. This conclusion was reached as far as tropical sprue was concerned from observations on only one case. 20 grammes of glucose were injected intravenously within 2 to 3 minutes in five patients with non-tropical sprue and one with tropical sprue. The curves in three of the six cases were found to be abnormally low and to fall rapidly to a fasting level—a finding which is stated to agree with the observations on the frequency of low blood sugar curves (50 per cent.) in their series after the oral administration of glucose. Some fifteen observations were also made on the respiratory quotient after the ingestion of 70 grammes of glucose in six cases of non-tropical sprue, three being described clinically as in a " good period " and three in a " bad period," one case of tropical sprue in a " good period " and one case of coeliac disease in a " bad period." This amount of glucose produced a rise in the respiratory quotient in his case of tropical sprue from 0·84–0·95 on one occasion and from 0·84–0·89 on another. Assuming the validity of so limited a series of observations in a group of patients some of whom were in remission and some in relapse, an elevation of the respiratory quotient merely shows that a certain amount—not necessarily all—of the ingested glucose is being absorbed and normally oxidized. FAIRLEY and BROMFIELD (1932) reporting the results of glucose tolerance tests in twenty-four cases of tropical sprue, regarded a rise of less than 30 mg. per 100 c.c. after 50 grammes of glucose as abnormally low. Fifteen out of twenty-four showed low values

according to this criterion, and, in addition, there was not infrequently a delayed sub-maximal rise in the blood sugar ; this was also sometimes observed even when the maximal exceeded 30 or 40 mg. per 100 c.c. They concluded that delayed absorption underlay the production of abnormal types of curves encountered in tropical sprue. Recently THAYSEN (1935) again elaborated his view that derangement of the regulation of blood sugar due to endocrine disturbance was the cause underlying flat oral glucose curves. No fresh data bearing on the question were recorded. HIMSWORTH (1934) suggested that a person with steatorrhoea would be very much in the same position as one on a high carbohydrate diet, since fat was not being absorbed and this would lead to a relative and probably an absolute increase in the amount of carbohydrate utilised. Since the ingestion of an excess of carbohydrate in the diet resulted in a flat type of sugar tolerance curve which was dependent, not on increased absorption of glucose, but on its increased rate of removal from the blood, HIMSWORTH saw no reason why the flat curve in idiopathic steatorrhoea should be brought about in a different fashion. These conclusions of course were based on the assumption that in steator-

GRAPH II.

BLOOD SUGAR CURVES IN SPRUE.

50 GRM. GLUCOSE IN 1 PINT OF WATER.
INTRAVENOUS INJECTION IN 15 MINS.

50 GRM. GLUCOSE PER OS.

rhoea glucose was rapidly removed from the blood as recorded by THAYSEN.
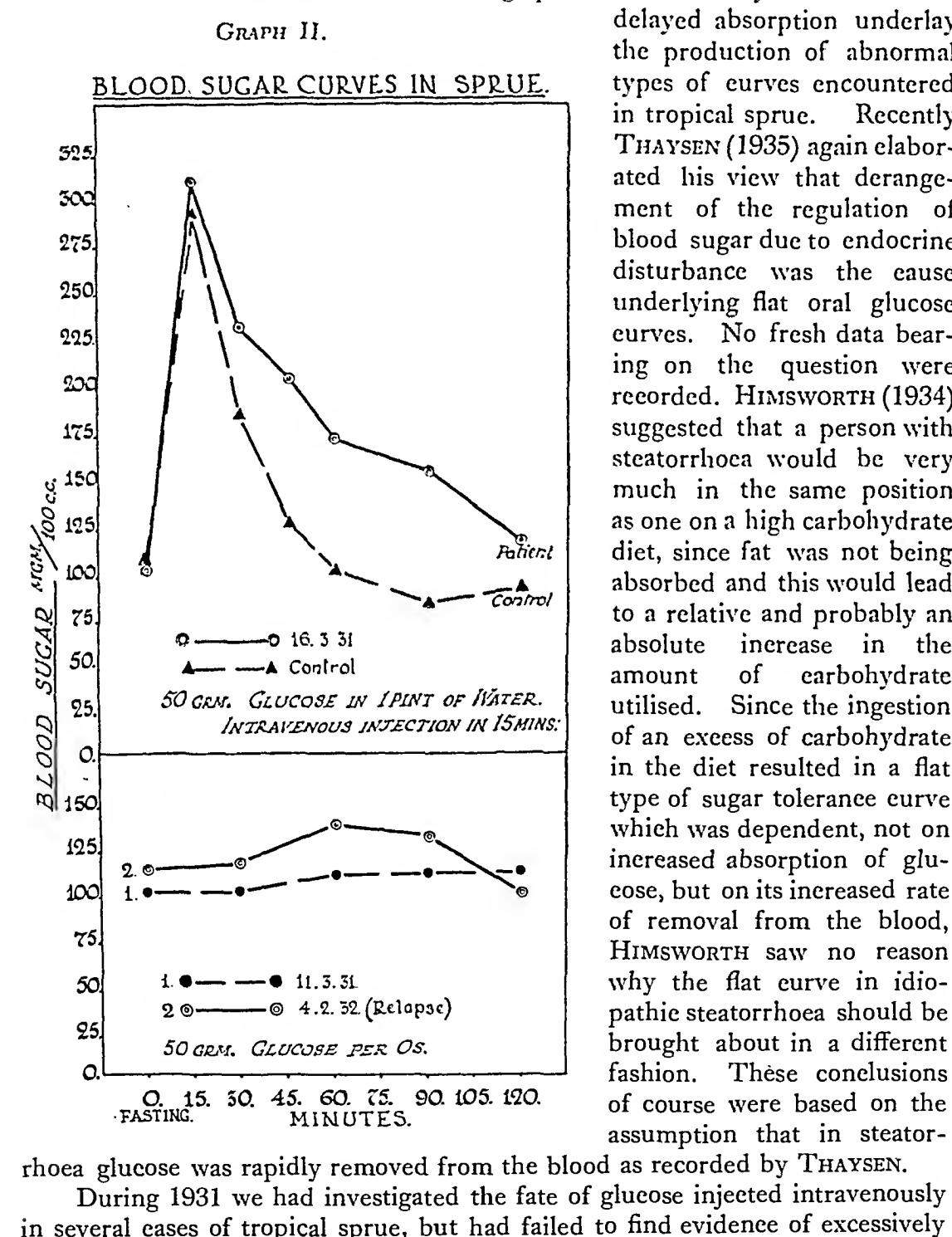
During 1931 we had investigated the fate of glucose injected intravenously in several cases of tropical sprue, but had failed to find evidence of excessively

rapid utilisation.   The results were not published at the time owing to the paucity of our observations, but in point of fact they were in complete accord with subsequent results.   Fifteen minutes was at that time taken for the intravenous injections in both control and sprue cases, and Graph II shows the type of curves obtained on oral and intravenous glucose in our first case to be investigated.   On admission (11/3/31) the curve was flat in type with a delayed maximal rise of only 13 mg. per 100 c.c. at 2 hours : the intravenous glucose curve showed a higher peak than normal and slow utilisation of glucose throughout the period of observation, judged by the high blood sugar values obtained compared with the control.   Subsequently (4/2/32) the patient returned to hospital with a mild sprue relapse and on this occasion a more normal oral curve was evident though the subnormal maximal rise only equalled 22 mg. per 100 c.c. at 1 hour.   After the correspondence by HIMSWORTH (1934) in the *Lancet* a further series of observations on the glucose tolerance curves following the oral and intravenous administration of glucose in cases of tropical sprue was undertaken, the period of administration being shortened to 10 minutes.

   (1) *Oral Glucose Curves.*—In Table 5 are epitomised the results of the blood sugar curve following the oral administration of 50 grammes of glucose in 50 cases of tropical sprue.   The average fasting blood sugar for the series equalled 100 mg. per 100 c.c.

TABLE 5.

BLOOD SUGAR CURVES IN TROPICAL SPRUE.
(Maximal rise after 50 grammes of glucose by mouth.)

Blood Sugar. mg. per 100 c.c.	Number of Cases.
0 – 10	7
11 – 20	14
21 – 30	11
31 – 40	6
Over 40	12

   It will be seen that in 38 cases (76 per cent.) the maximal rise was under 40 mg. per 100 c.c. which is the standard now taken as subnormal.   In twelve cases (24 per cent.) it exceeded 40 mg. per 100 c.c., but in four of these the

maximal rise was delayed until $1\frac{1}{2}$ hours or later. Thus forty-two cases or 84 per cent. presented abnormal oral glucose curves on the first examination.

Furthermore, as patients improved under treatment it was found that flat and low curves were replaced by higher curves and that they not infrequently passed through a stage with a delayed maximal rise before finally returning to a normal contour. This is well illustrated in Graph III. The flat curve (1)

GRAPH III.

ORAL GLUCOSE TOLERANCE CURVES IN A CASE OF SPRUE PRIOR TO, DURING AND AFTER TREATMENT.

Note the transition from a curve to flat type (1) to ones showing delayed maximal rises (2) and (3) and finally to a normal curve (4).
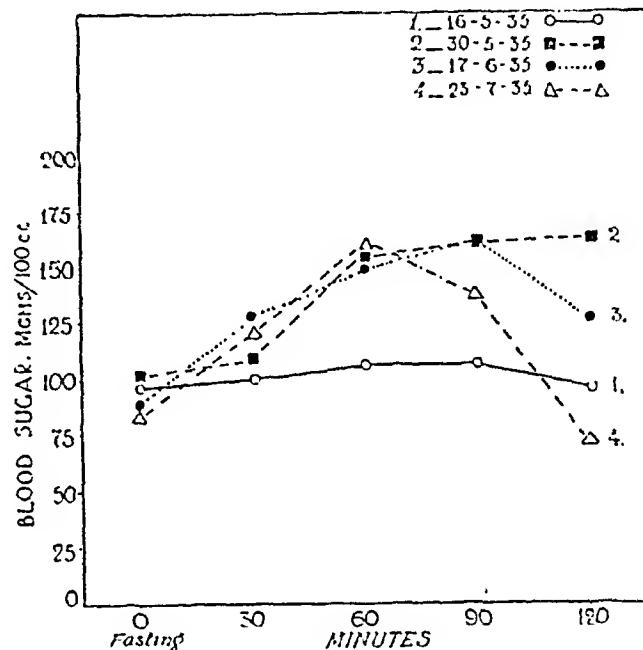
with a maximal rise of 10 mg. per 100 c.c. was present on admission ; after 14 days treatment with high protein, low fat, low carbohydrate diet and liver extract *per os* this was replaced by curve (2) in which there was a delayed maximal rise of 59 mg. at 2 hours ; 32 days later the maximal rise of 70 mg. (3) was observed at $1\frac{1}{2}$ hours ; by 68 days a normal glucose tolerance curve (4) had been established.

Such transitional types of curves as (2) and (3) in Graph III, are of special interest since they throw an interesting side-light on the probable significance of oral curves with a delayed high maximal rise which are occasionally encountered in elderly patients with severe sprue in whom intravenous glucose tolerance curves reveal a marked degree of decreased sugar tolerance. The curves from such a patient are depicted in Graph IV. Here blood sugar is being less rapidly utilised than normally and presumably less efficiently absorbed as well. Certainly the clinical features presented by such patients suggest this to be so. In old people the oral glucose tolerance curves tend to be high, and probably their glucose tolerance is somewhat decreased compared with younger age groups.

GRAPH IV.

ORAL GLUCOSE CURVE IN A CASE OF TROPICAL SPRUE SHOWING A DELAYED HIGH MAXIMAL
RISE ASSOCIATED WITH SLUGGISH UTILISATION OF BLOOD SUGAR,
i.e., HIGH TYPE OF INTRAVENOUS CURVE.

TROPICAL SPRUE.
GLUCOSE CURVES on ADMISSION (SPRULAC Nº1 Diet).
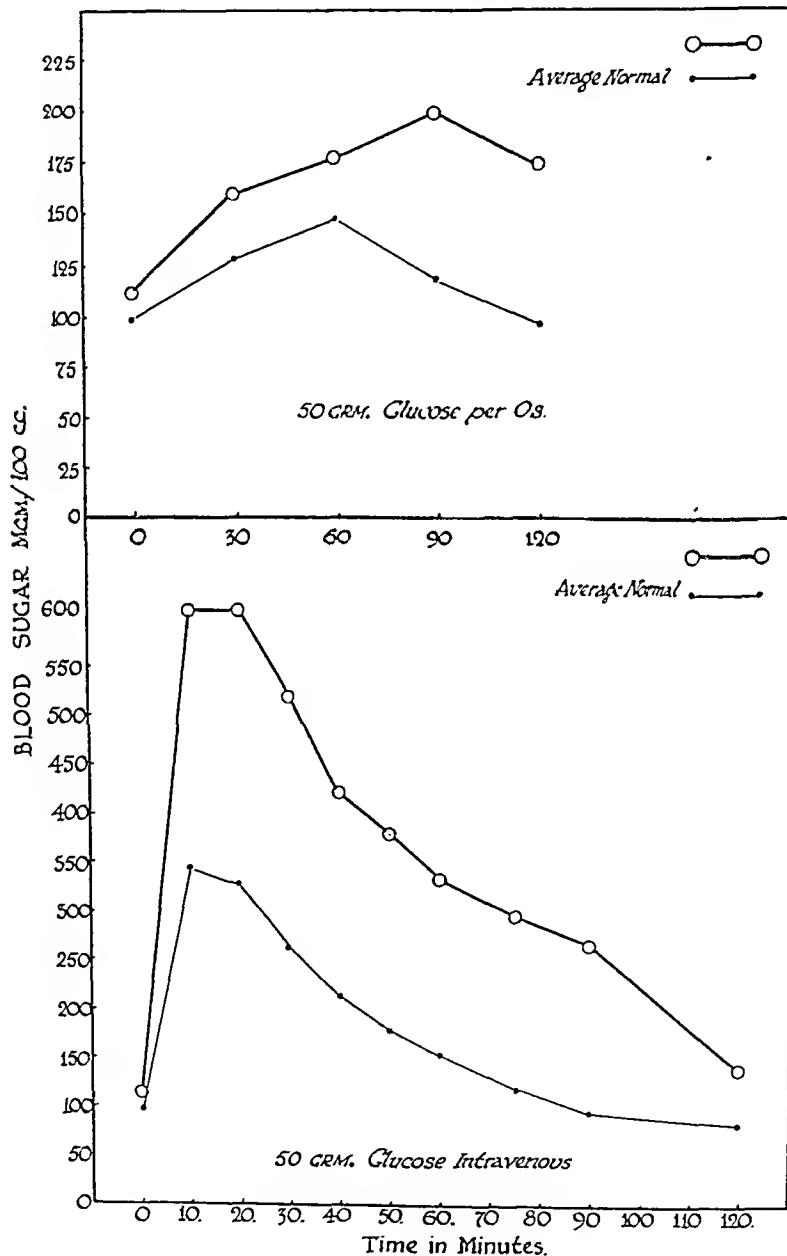
Graph V shows another type of oral curve taken on admission (11/7/35) which showed a delayed subnormal maximal rise of 18 mg. per 100 c.c. at 1½ hours; 1 week later (18/7/35) a high intravenous curve indicative of decreased glucose tolerance was demonstrated. On this occasion there was some slight delay so that the injection of glucose took 15 instead of the usual 10 minutes. Some 25 days after treatment with high protein, low fat, low carbohydrate diet and liver extract *per os* the patient had made remarkable clinical progress. On repeating the oral glucose tolerance test 26 days later a normal shaped glucose curve with a subnormal maximal rise of 35 mg. per 100 c.c. at 1 hour was found. The intravenous glucose curve was not repeated.

(2) *The Intravenous Glucose Tolerance Test.*— The technique was finally standardised so that the individual received 50 grammes of glucose intravenously in 1 pint of distilled water within 10 minutes on a fasting stomach. In selecting cases for con-

GRAPH V.

ORAL GLUCOSE CURVES IN SPRUE SHOWING A DELAYED MAXIMAL RISE (1) WITH IMPROVEMENT ON TREATMENT (2). THE INTRAVENOUS GLUCOSE CURVE REVEALED DECREASED SUGAR TOLERANCE.

trols only those individuals presenting a normal type of oral glucose curve were utilised. Blood sugar estimations were made at 10 minute intervals

up to 120 minutes and the results plotted in the usual fashion. Biochemical data collected from a series of normal individuals and typical cases of tropical sprue are epitomised in Table 6.

TABLE 6.

INTRAVENOUS GLUCOSE CURVES IN TROPICAL SPRUE.
(Ten Cases.)

Time.	Blood Sugar mg. per 100 c.c.	
	Average in Normal Controls.	Average in Tropical Sprue.
10 minutes*	344	389
1 hour	137	235
1½ hours	93	178
2 hours	84	130

*All cases received 50 grammes of glucose intravenously within 10 minutes. The maximal rise occurred at this time.

Throughout the period of observation the curve of blood sugar in tropical sprue showed values higher than the normal. The average maximal rise exceeded the average normal control by 45 mg. per 100 c.c. at 10 minutes, by 98 mg. at 1 hour, by 85 mg. at 1½ hours and by 46 mg. per 100 c.c. at 2 hours, by which time the blood sugar had not returned to the fasting level. The results may be more clearly appreciated by a perusal of Graph VI. At a later date it is hoped to publish a standard normal curve with maximal and minimal variations. In the meantime it may be stated that the individual maximal curve in each of these ten cases of sprue was higher than the upper maximal curve encountered in any of our normal series. The intravenous curves have been so consistently high in sprue cases that the data obtained entirely support the view that in tropical sprue prior to treatment, flat oral curves, curves with a subnormal maximal rise and curves with a delayed normal maximal rise are associated with high intravenous curves indicative of decreased glucose tolerance. These findings are in complete disagreement with those of THAYSEN and are incompatible with the view held that rapid removal of sugar from the blood, either by endocrine disturbance (THAYSEN) or by compensatory excessive carbohydrate intake (HIMSWORTH), is responsible for the abnormal types of oral glucose curves encountered in tropical sprue.

GRAPH VI.

INTRAVENOUS BLOOD SUGAR CURVES IN SPRUE.
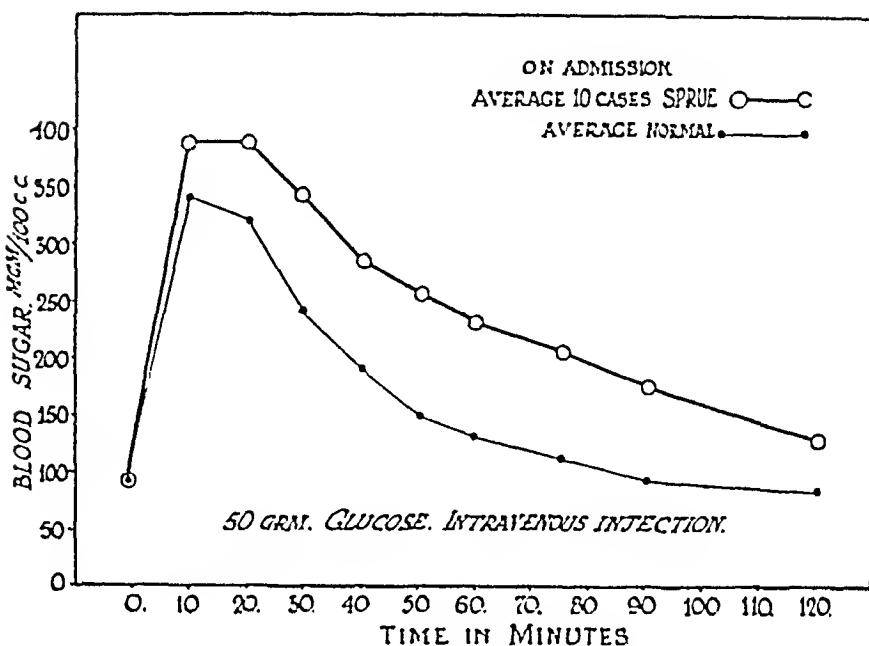
Effects of Treatment.—Reference has already been made to the effects of successful treatment on the abnormal types of oral glucose curve and to the gradual restoration to a normal contour as intestinal signs and symptoms disappear and recovery ensues. In the limited number of cases investigated corresponding changes have been encountered with the intravenous curves, only here high curves give place to lower curves which gradually approximate to the normal. Graph VII illustrates the effects on the sugar tolerance of treatment with combined high protein, low fat, low carbohydrate diet and oral liver extract therapy in a patient with tropical sprue of 2 years duration. On admission (14/2/36) the oral curve was almost a straight line, a maximal rise of 10 mg. per 100 c.c. being recorded at 1 hour. Three days later (17/2/36) the intravenous curve was found to be consistently higher than the normal average curve, indicating a state of decreased glucose tolerance. Next day (18/2/36) treatment was started. Forty-three days later (31/3/36), by which time the patient was free from abdominal symptoms and the red cell count had risen from 2,315,000 to 4,830,000 per c.mm , the oral glucose curve showed a maximal rise of 53 mg. per 100 c.c. at 1 hour. By the 53rd day the intravenous curve was closely approximating to the normal. Graph VIII illustrates an even more complete restoration of the intravenous glucose curve to normal limits in a patient who had suffered from sprue for 10 years. The oral glucose test gave a rise of only 8 mg. per 100 c.c. at 1 hour interval (6/12/35). The intravenous tolerance test performed 5 days

GRAPH VII.

BLOOD SUGAR CURVES IN SPRUE BEFORE AND AFTER TREATMENT.
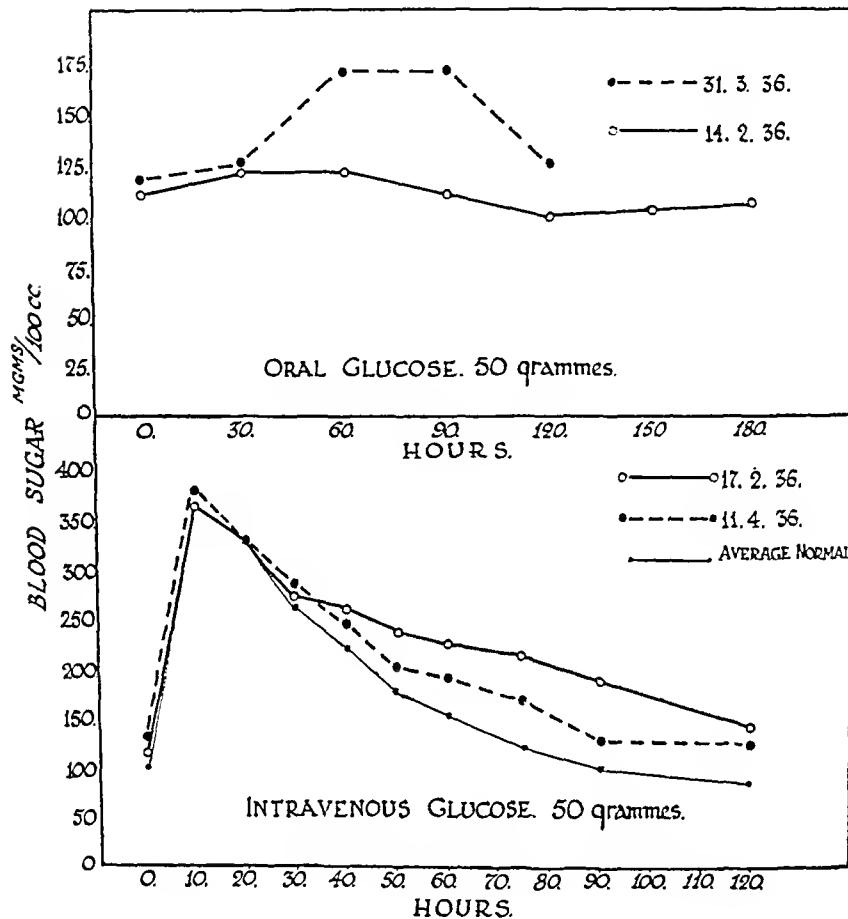
later, showed higher values than the normal control, indicating a mild degree of decreased glucose tolerance. Treatment commenced the same day (11/12/35). Another oral curve 41 days later showed a maximal rise of 49 mg. per 100 c.c. at 1 hour. The following day the intravenous tolerance curve revealed values slightly below the average normal. Restoration of glucose tolerance coincided with clinical recovery.

It has only proved possible to repeat the intravenous curves prior to discharge from hospital in four of the ten cases of the series, and further data are being collected on this point, but already it is evident that with clinical cure and restoration of intestinal function the intravenous no less than the oral glucose tolerance curves attain normal limits.

GRAPH VIII.
TROPICAL SPRUE.
RESTORATION TO NORMAL OF THE INTRAVENOUS GLUCOSE TOLERANCE CURVE IN SPRUE FOLLOWING EFFECTIVE TREATMENT.
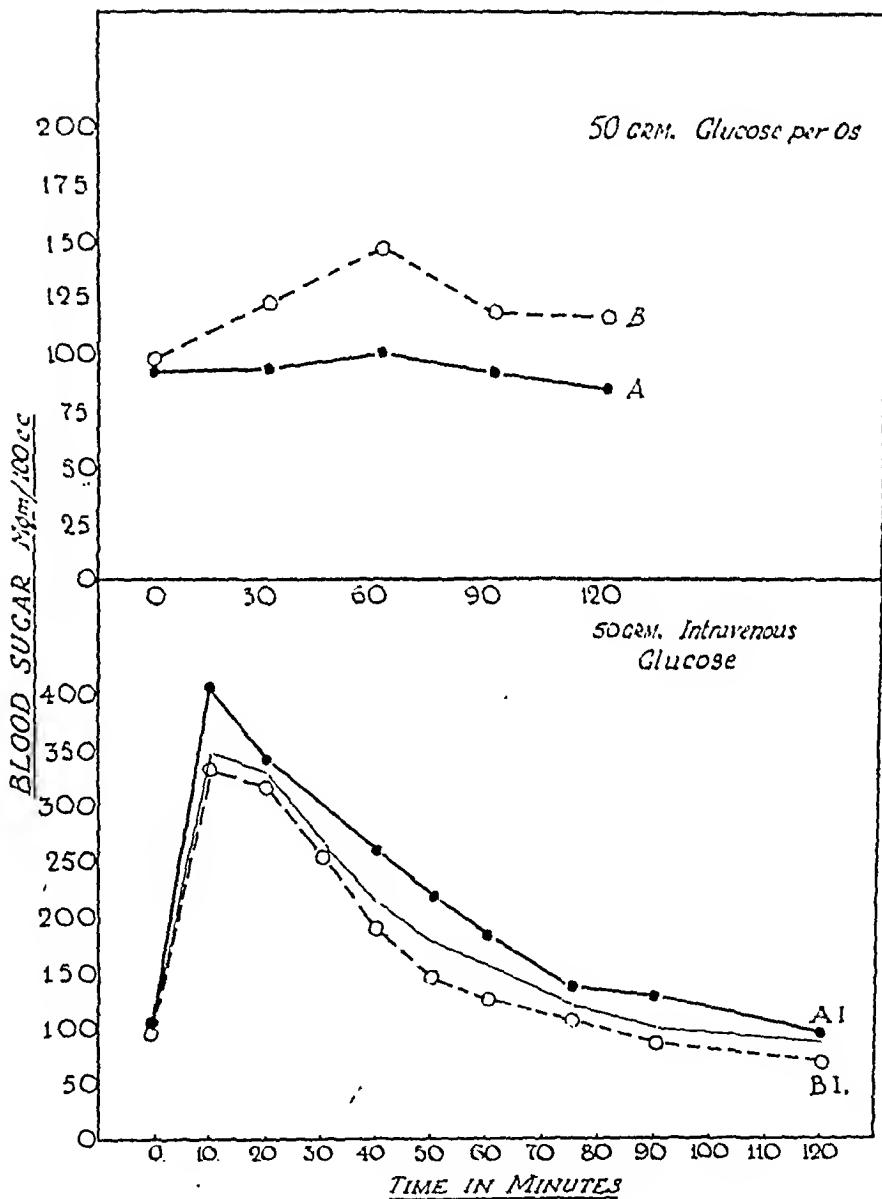
In Graph IX will be found glucose tolerance curves in a female patient aged 73 years who had repeatedly suffered from relapses of tropical sprue. The first oral curve prior to treatment (28/1/36) showed a delayed maximal rise of 63 mg. per 100 c.c. at 1½ hours associated with a high intravenous curve indicative of decreased sugar tolerance (24/1/36).

After intensive treatment with campolon (6 c.c. daily) and sprulac and subsequently liver extract *per os* the blood was restored to normal values, and

GRAPH IX.

TROPICAL SPRUE.

GLUCOSE CURVES ON ADMISSION AND AFTER TREATMENT.
HAEMATOLOGICAL RESPONSE MORE SATISFACTORY THAN
INTESTINAL. CLINICALLY NOT CURED.
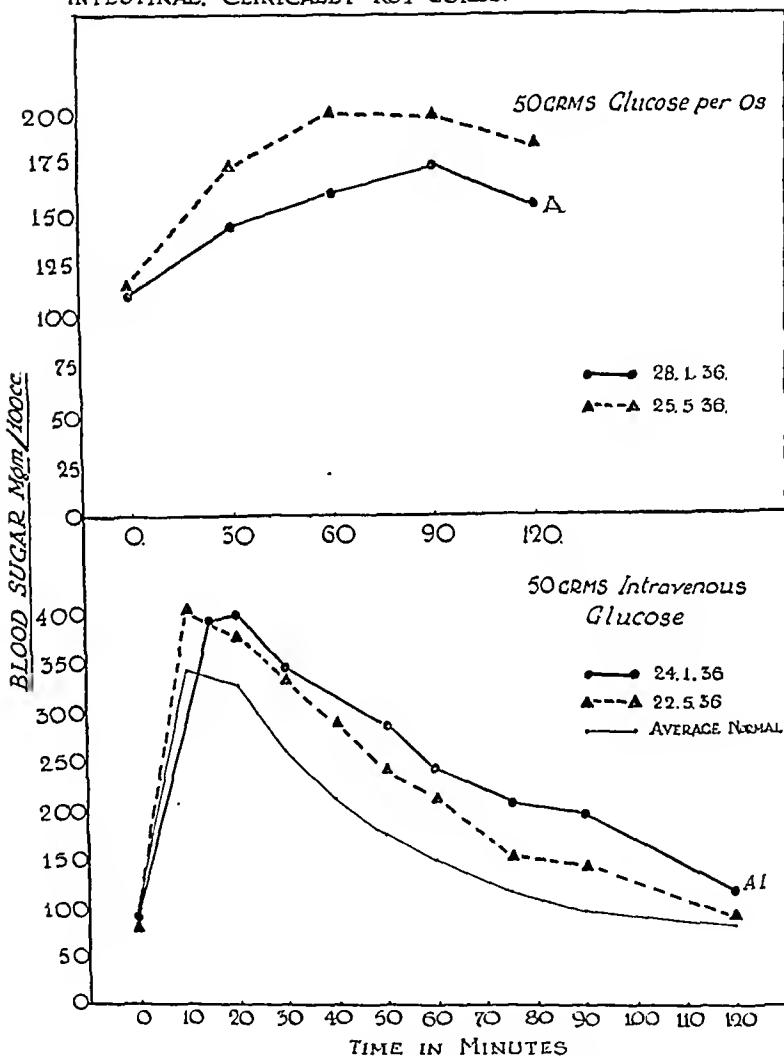
the nutrition and clinical condition of the patient greatly improved. On retesting (25/5/36) the oral glucose curve showed a delayed maximal rise of 87 mg. per 100 c.c. at 1½ hours, associated with an intravenous curve (22/5/36) which though still high was considerably lower than the one taken 4 months previously. The administration of glucose on this occasion unfortunately precipitated a mild relapse with abdominal distension, much intestinal flatulence and some looseness

of the bowels indicating that the patient though clinically vastly improved had not been completely cured.

(3) *Commentary.*—The explanation of flat oral glucose curves associated with high intravenous ones is to be found in defective absorption of glucose which is the equivalent of carbohydrate deprivation. This, as HIMSWORTH has shown, results in decreased sugar tolerance dependent on a decreased sensitivity to endogenous insulin.* Ketonuria is not demonstrable in these cases, a finding due to the gradual onset and chronic nature of the carbohydrate deprivation to which the body soon adapts itself. A similar adaptation is noted in patients placed on a ketogenic diet.

From a dietetic viewpoint intestinal flatulence and abdominal distension are best controlled by strictly limiting the carbohydrate moiety of the diet, just as loose, bulky, greasy stools, hypocalcaemia and tetany are best controlled by decreasing its fat content. One of the difficulties encountered in the oral tolerance test is the tendency for the sprue patient to develop acute abdominal distension and looseness of the bowels after taking the requisite amount of glucose (50 grammes). It is the abnormal fermentation of unabsorbed glucose, not of starch, which is directly responsible for the intestinal flatulence, abdominal distension and the gaseous, acid features of the stools. The increased acidity favours the growth of yeasts, especially of *Monilia ashfordi*, to which for many years ASHFORD ascribed a primary aetiological role. That starch digestion is not affected is indicated by the absence of any excess of undigested starch granules in the faeces, as previously noted.

(d) Protein Metabolism.

Clinical experience in the value of high protein dietary in the treatment of tropical sprue entirely supports the view that ingested protein is utilised normally, and this is confirmed by the available biochemical evidence. THAYSEN (1932) recently summed up the literature on the subject and concluded that the loss of nitrogen by way of the stools during periods of fatty diarrhoea does not as a rule exceed the normal, and that where it does, the increase in faecal nitrogen output comes from increased intestinal secretion, not from malabsorption. Similarly, the non-protein and protein-nitrogen content of the blood are generally normal. FAIRLEY, MACKIE and their colleagues (1926) reported normal values for the non-protein nitrogen in seventeen cases of tropical sprue (average = 35·6 mg. per 100 c.c.) ; and average values within normal limits for albumin (4·69 g. per 100 c.c.), globulin (1·92 g. per 100 c.c.) and fibrin (0·368g. per 100 c.c.) in nineteen cases. In tropical sprue oedema in the absence of renal and cardiac complications occasionally occurs. The association of low plasma proteins and hypo-cholesterolaemia would suggest a nutritional origin dependent on defective absorption of protein from the intestine. Such a condition must be very rare in tropical sprue.

*It naturally follows that injections of intravenous glucose solution and insulin are both indicated in severely ill sprue patients with decreased glucose tolerance.

(e) Vitamin Deficiency.

A study of the dietaries of our patients at the time they developed sprue fails to support the view of ELDERS (1919) and others that avitaminosis is a primary factor in the aetiology of the disease. Vitamin deficiency, secondary to malabsorption or following restrictions of diet imposed later with the object of cure, is a possibility, and certain of the clinical manifestations may be explicable on this basis. The relationship of fat-soluble Vitamin D to the genesis of hypocalcaemia and tetany has already been considered, but there is no evidence that the other fat soluble Vitamins A and E are implicated in tropical sprue. Similarly, the mild neuritic features encountered in tropical sprue may be due to defective absorption of Vitamin B₁ and some have attributed the occasional incidence of oedema to the same cause. The infrequent purpuric manifestations may likewise be explained on the basis of a Vitamin C deficiency. These features, however, are much less pronounced than in either coeliac disease or idiopathic steatorrhoea and constitute further evidence of the milder nature of the intestinal breakdown in tropical sprue.

[*Part II of this paper will appear in a subsequent number of the* TRANSACTIONS.]

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INTESTINAL ABSORPTION IN COELIAC DISEASE, WITH SOME REMARKS ON THE EFFECT OF LIVER EXTRACTS UPON CARBOHYDRATE METABOLISM.

BY

C. WALLACE ROSS, M.B., B.S. (MELB.),*

Resident Medical Officer, Children's Hospital, Birmingham.

The recognition of coeliac disease as a condition of disordered absorption lagged many years behind its first masterly clinical description by SAMUEL GEE in 1888. In fact his surmise that the intestinal phenomena were possibly due to " a kind of chronic indigestion " was not pressed further until CHEADLE (1903) put forward his theory of " acholia." In the past 20 years, however, a great deal has been added to our knowledge of the disease, and the whole of this new information was admirably summarised up to 1932 by Professor PARSONS (1932) in his Rachford Memorial Lectures. This critical and constructive survey is so well known that it is unnecessary here to do more than enumerate, with the briefest of comment the absorptive deficiencies at present recognised, before proceeding to a more detailed consideration of the question of carbohydrate absorption.

Fat.—Inability to absorb fat and the products of its digestion is, of course, the keynote of the disorder, and there is nothing new to be said on the subject. PARSONS laid down the guiding principle that a daily output of more than 2 grammes of fat (usually found where the dried faeces contain more than 25 per cent.) should be required before the diagnosis is made ; and this rule has proved most valuable in that it agrees closely with one's clinical conception of the border-line of disease.

C

Protein.—With regard to protein there is little doubt that during periods of diarrhoea the absorption is as poor as it is with diarrhoea of any aetiology. But the figures obtained by PARSONS and HICKMANS on four cases show that in non-diarrhoeal periods from 85 to 95 per cent. of the nitrogenous intake can be absorbed. This conclusion is fully supported by the fact that many cases are carried through successfully on diets which would be quite inadequate unless most of the protein were absorbed.

Vitamin A.—Xerophthalmia is a great rarity in coeliac disease, having been noted twice only—by SCHICK (1929) and by FANCONI. There is, however, a strong clinical impression that these children are much more subject than others to low-grade skin infections, coryza and otorrhoea, often accompanied by parenteral diarrhoea ; and this may possibly be related to deficiency of Vitamin A.

Vitamin B.—In a few cases oedema occurs, sometimes even frank ascites, without obvious cause. Marked improvement in such conditions has been seen by HAAS (1929) and PARSONS following the use of ripe banana or marmite, and it is thought that this oedema may conceivably represent infantile beri-beri. Confirmation of such a view by nervous signs is scanty, except for the occasional absence of knee jerks. Tenderness of the limbs is often present—even in rare cases pseudo-paresis may be seen—but most observers agree that these are more likely to be scorbutic manifestations.

Vitamin C.—Scurvy is, in fact, by no means a rare complication. Usually it is not severe, but it may occasionally lead even to haemorrhage from the bowel.

Vitamin D, Calcium and Phosphorus.—As one must expect, the steatorrhoea leads to a heavy loss of calcium and to great difficulty in absorbing Vitamin D. Further, unless attention is directed to the matter, the specially low fat diets used in treatment will still further limit the usual intake of the fat-soluble vitamin. It is not surprising, then, that rickets and tetany should be common, though of very varying geographical distribution, while some degree of osteoporosis is almost inevitable. Low blood calcium and phosphorus values are frequently found.

Iron.—Most cases of coeliac disease show a mild degree of anaemia of the hypochromic type, responding easily to treatment with iron. Only occasionally are severe degrees of this encountered.

Essential Factor.—As a great rarity in long-standing cases a macrocytic anaemia may be found.*

CARBOHYDRATE.

I shall now leave this wide field of absorptive deficiencies to give closer attention to the question of carbohydrate absorption which is more particularly the subject of this paper. The idea that the absorption of carbohydrate is

* Vide remarks by Professor L. G. PARSONS in opening the discussion.

deficient in coeliac disease is no new one, and the importance in treatment of controlling the intake was strongly emphasised by HOWLAND (1921). The evidence on which these early clinical convictions were based included the fermentative character of the diarrhoea in many cases (the stools being frothy and of acid reaction), the great flatulent distension of the colon and the response of these symptoms to control of the carbohydrate in the diet.

Later MACLEAN and SULLIVAN (1929) and THAYSEN and NORGAARD (1929), together with ELISABETH SVENSGAARD (1930), showed that oral glucose tolerance tests in these cases gave flat curves—a finding with which all workers are in agreement. It is in the interpretation of these curves that much disagreement has arisen.

MACLEAN and SULLIVAN eliminated the possibility of a lowered renal threshold, and finally held that an endocrine defect offered the most likely explanation—a view with which THAYSEN agreed.

MARFAN (1929) and PARSONS (1932) preferred to regard the disorder as an absorptive one ; but as carbohydrate balance experiments by faecal analysis are not possible, no positive proof could be given.

THAYSEN (1935)—whose recent death removes one of the most active workers from this field—strongly opposed this conception. His reasons were three in number :—

1. " Following intravenous injection of glucose, patients with flat blood sugar curves show a flatter curve than do normal persons."
This finding is the reverse of my own.

2. " After the administration of glucose by mouth the respiratory quotient rises to one or to about one."

I have done no work on this point, but venture to suggest that there is little reason for placing great weight on the evidence of respiratory quotient determinations, unless it is fully supported.

3. " On a diet rich in carbohydrates the respiratory quotient is higher than on a mixed diet, as in the case of normal persons."

This does not seem to demonstrate that there is *no* defect in absorption, for such a change might well follow if the higher diet led merely to a slight quantitative improvement in intake.

For some time then these two opposed views, of an endocrine or an absorptive defect, held the field. In the last few years, however, there has occurred a great extension and simplification of our knowledge of carbohydrate metabolism with a renewal of interest in this subject. It is now possible to state three simple but comprehensive axioms concerning glucose tolerance :

1. The tolerance of an individual for glucose is directly proportional to the sensitivity to injected insulin. The relation of the two properties is in fact

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1. The tolerance of an individual for glucose is directly proportional to the sensitivity to injected insulin. The relation of the two properties is in fact

a linear one, and glucose tolerance may be regarded as sensitivity to the endogenous insulin.

2. Insulin sensitivity is due to the presence in the body of a third factor in the glucose-insulin reaction, now usually referred to as " insulin-kinase."

3. The degree of insulin sensitivity of a healthy individual and hence the glucose tolerance, is determined solely by the amount of carbohydrate the diet has contained for the period preceding the test.

HIMSWORTH (1934), to whose classical work much of this simplification is due, offered a third explanation of the low oral curves in coeliac disease, based on the third of these axioms. He argued that, in view of their inability to absorb fat, coeliac patients must be living largely on carbohydrate, and hence might be expected to show an increased glucose tolerance, reflected in a low oral curve.

It seemed to me that, with the aid of the new knowledge of glucose tolerance, decisive evidence might at last be sought as to the presence or absence of an absorptive defect, the principle of the experiment being as follows.

In the normal subject obviously carbohydrate privation will lead to impaired glucose tolerance, which will be shown by a high and rather slowly-falling blood sugar curve following the administration of glucose, whether by the alimentary tract or intravenously ; while carbohydrate excess will lead to an opposite condition of improved tolerance, with a low curve in both cases. Such a series of curves obtained from a healthy boy of 5 years is shown in Figs. 1 and 2.
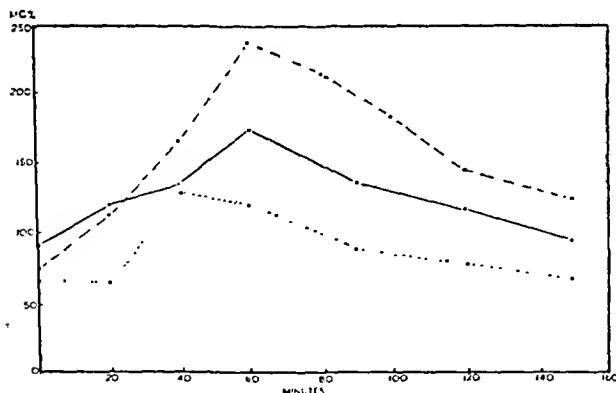
FIG. 1.—Oral glucose tolerance curves obtained from a healthy boy of 5 years after giving 30 grammes of glucose by mouth (solid line, when on normal diet ; dashed line when on low carbo-hydrate diet ; dotted line when on high carbohydrate diet).

In the presence of any disease process such as would hinder or prevent the absorption of glucose from the gut, one would expect a different state of affairs, for here the oral curve should be of the flat type from failure of sugar to reach the blood, while the intravenous one should be high as a result of the same intolerance as that which would follow carbohydrate deprivation in a normal person.

Accordingly it was decided to compare the oral and the intravenous glucose

FIG. 2.—Intravenous glucose tolerance curves obtained from a healthy boy of 5 years after injecting 10 grammes of glucose (solid line, when on normal diet; dashed line when on low carbo-hydrate diet; cross-hatched line when on high carbohydrate diet).
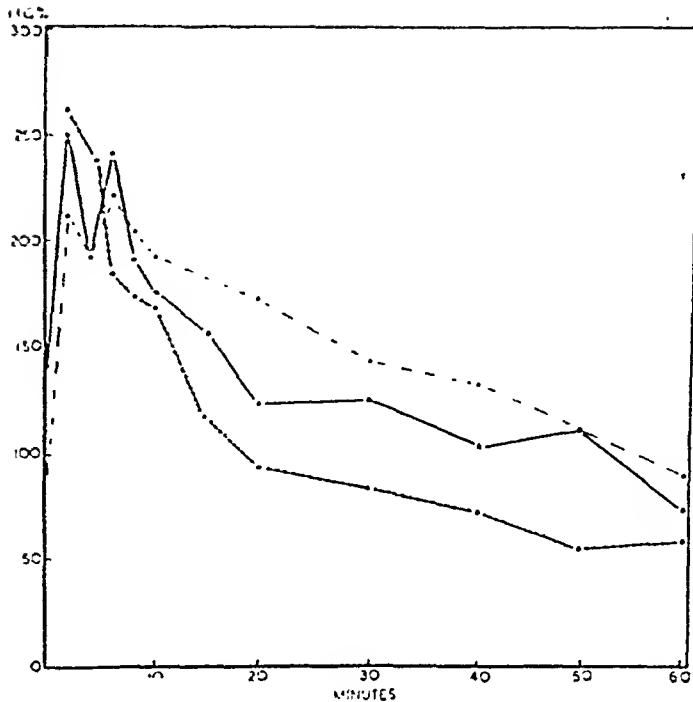
FIG. 3.—Intravenous glucose tolerance curves in three cases of coeliac disease (upper group of curves) contrasted with a com-parable normal curve (cross-hatched).
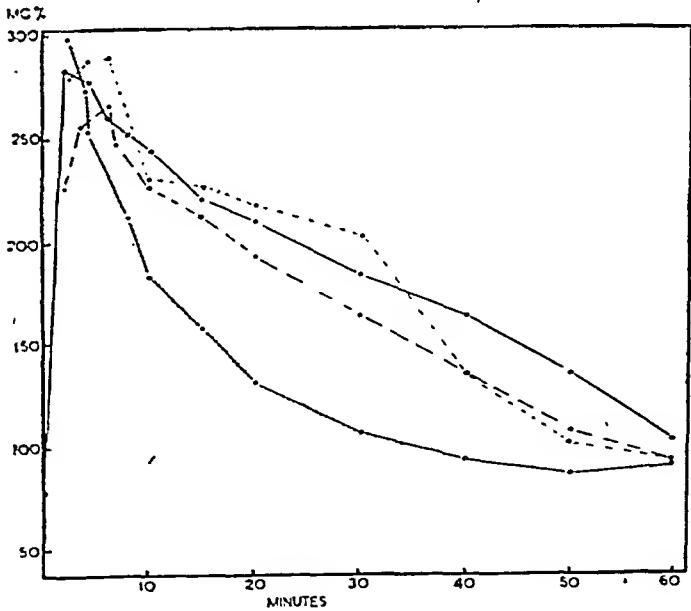
tolerance curves of a group of cases. The result was a striking one indeed, for the intravenous curves (Fig. 3) were as consistently high, that is to say indicative of impaired tolerance, as the orals (Fig. 4) were flat. In Fig. 5 are shown curves indicating the mean elevation of the blood sugar above fasting level in ten cases of coeliac disease (dashed line) and in four normal children
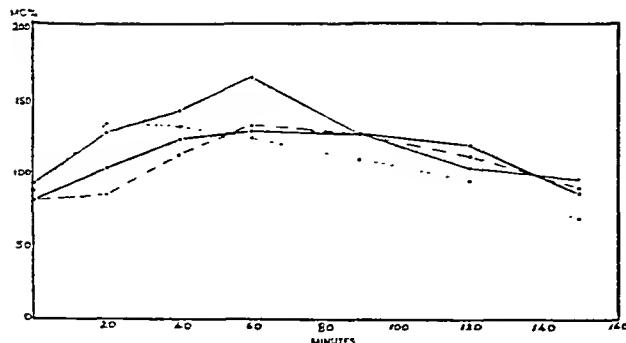
FIG. 4.—Oral glucose tolerance curves in three cases of coeliac disease (lower group) contrasted with a comparable normal (highest line).
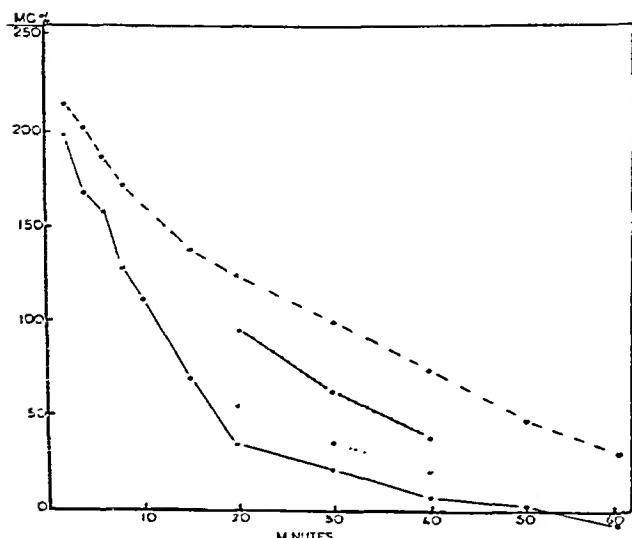
FIG. 5.—Curves showing the mean elevation of blood sugar levels after giving 10 grammes of glucose intravenously to ten cases of coeliac disease (dashed line) and to four comparable normal cases (solid line). Sections of the lowest coeliac curve (cross-hatched line) and of the highest normal (dotted line) are included to show that there is, in the 20 to 40 minute period, no overlapping of curves.

of similar age (solid line). Not only is there a sharp contrast between these means, but in the 20 to 40 minute period there is no overlapping of the curves of the two series of cases. To demonstrate this, a section of the lowest coeliac curve is shown by a cross-hatched line, and a similar section of the highest normal by a dotted one. This result seems to offer strong evidence for the existence of an absorptive defect.

Further, if this is so, and if privation of carbohydrate by failure of absorption is the sole reason for the impairment of tolerance observed when a test dose of glucose is given intravenously, then the tolerance should be restored towards normal by supplying the body with glucose by the intravenous route over a suitable period of time. Opportunity to demonstrate this change was afforded me in the case of a little girl, M.K., of 22 months, who was admitted to hospital with the history that she had been well until 5 months previously when the stools became large, pale, often slimy and rather frequent. She began to lose weight and the abdomen became noticeably large, while the buttocks underwent a

diminution in size. The faeces showed a daily output of 16·5 grammes of total fat (73·1 per cent. of the dried faeces collected over 5 days).

FIG. 6.—Oral glucose tolerance curves in the case (M.K.) of coeliac disease, before treatment (solid line) and in a later period of remission (dashed line).
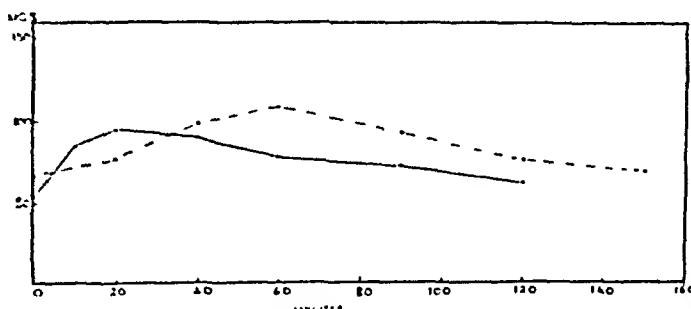
An oral glucose tolerance test at this time gave (Fig. 6 solid line) the following result (15 grammes as 20 per cent. solution) :—

	Fasting	10	20	40	60	90	120 minutes.
Mg. per cent.	54	84	96	90	78	72	61

An intravenous glucose tolerance test (10 grammes as 20 per cent. solution injection 2 minutes) showed a type of high curve which we find usually in severe cases (Fig. 7 short-dashed line) :—

	2	4	6	8	10	15	20	30	40	60 minutes.
Before injection	235	276	249	235	216	242	258	223	153	129 106
Mg. per cent. 52										

Having improved considerably she was discharged in 17 days to carry on treatment at home. This was not successful and 6 weeks later she was re-admitted in a very poor condition, having diarrhoea of a gross degree. Treatment along the usual lines followed for some 10 days. By this time dietary measures had controlled the diarrhoea but the child was desperately weak and vomiting once or twice a day. In the belief, founded partly on her previous low fasting blood sugar levels, that hypoglycaemia was the cause of much of the trouble, continuous intravenous treatment with glucose solution was decided upon. This was initiated by an intravenous glucose tolerance test, at the conclusion of which 63 grammes of glucose were run in over 43 hours, in the form of a 10 per cent. solution in normal saline. Five hours after the completion of this, a further intravenous glucose tolerance test was carried out. Fig. 7 shows the curves obtained on this occasion, as well as that done at her first admission. The clinical result of the treatment was a dramatic improvement, the credit for which must probably be shared with a blood transfusion which immediately followed. It is interesting to note that, after having whimpered ceaselessly for days when not asleep, the child lay completely at peace after the first 10 gramme dose of glucose, saying she was " bett." Five weeks later a similar emergency arose and the same line of treatment was adopted. The tests done on this occasion showed almost identical results, and the clinical improvement was again most gratifying.
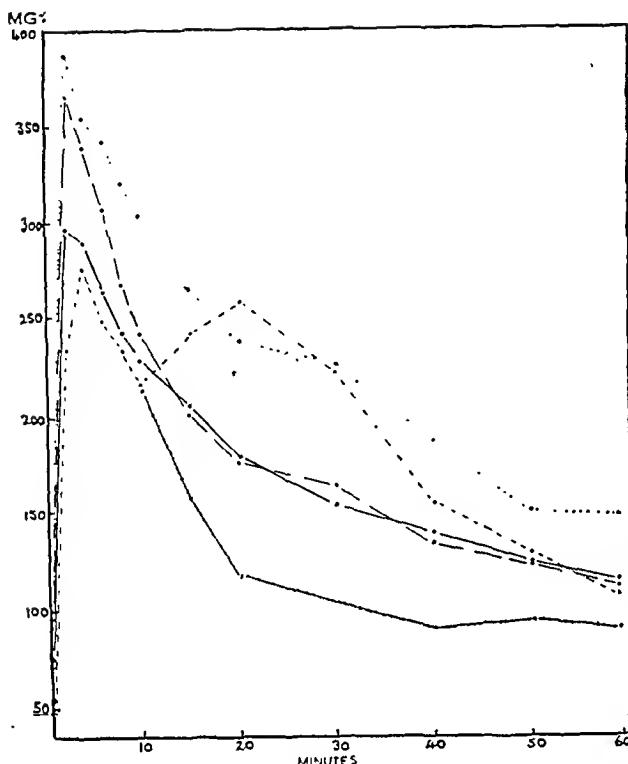
FIG. 7.—Intravenous glucose tolerance curves in the case (M.K.) of coeliac disease :—

1. At diagnosis and before treatment (short-dashed line).

2. During exacerbation, before intravenous glucose therapy (dotted line).

3. After intravenous glucose therapy (long-dashed line).

4. After prolonged treatment by campolon injections (solid line).

A portion of a comparable normal curve is shown as a cross-hatched line (lowest).

Two months later her condition was so far improved that an oral glucose tolerance test could be carried out with reasonable safety. Its result (Fig, 6, dashed line) is noted, lest the diarrhoea existing at the time of the previous oral test should be put forward as the cause of impaired absorption. The dose was as usual 30 grammes given as 20 per cent. solution and the figures obtained were :—

	Fasting	20	40	60	90	120	150 minutes.
Mg. per cent	69	78	99	108	92	75	69

So far then, I have demonstrated firstly the association of high intravenous glucose tolerance curves with the flat oral ones which are generally recognised, and secondly the possibility of reducing these high intravenous curves towards normal by giving glucose intravenously over a period of time. Let us now turn to the confirmatory evidence to be derived from a study of the insulin depression curves. For permission to use a large number of his results in this connection I am greatly indebted to Dr. A. V. NEALE.

BADENOCH and MORRIS (1936) have recently published results showing, in their own words, that " children with coeliac disease are more sensitive to the effect of insulin than are non-coeliac patients, as shown by the greater percentage reduction of blood-sugar in the former after injection of insulin." With this

conclusion I am quite unable to agree, the whole of our observations going to show the reverse.

Fig. 8 shows three typical insulin depression curves of our series. These were obtained by the injection of four units of crystalline insulin (B. W. & Co.) solution into a vein of the fasting child, the blood-sugar being estimated at short intervals thereafter.
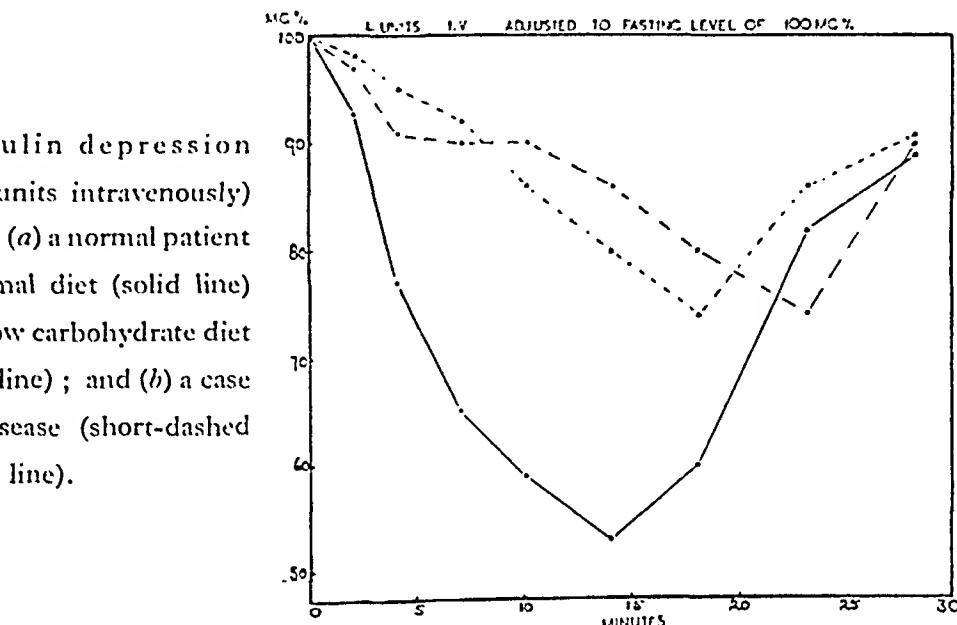
FIG. 8.—Insulin depression curves (four units intravenously) obtained from (a) a normal patient (1) on a normal diet (solid line) and (2) on a low carbohydrate diet (long-dashed line) ; and (b) a case of coeliac disease (short-dashed line).

The curve represented by a solid line is that of a normal patient on a normal diet. That indicated by long dashes was derived from the same patient after a week on a low carbohydrate diet, while that indicated by short dashes represents a typical member of our coeliac series, not on a low carbohydrate diet and in a period of freedom from diarrhoea.

In Fig. 9 I have graphed the maximal depression obtained in all of Dr. NEALE's cases, with some other observations. The plain dots represent in the upper group coeliacs, in the lower normals. The two dots joined by a line represent the initial state of a case of coeliac disease at diagnosis, and the improvement after 7½ months' treatment. That enclosed in a diamond was obtained from a case, now aged 12½ years, which has been under treatment from the age of 20 months, and has now practically no steatorrhoea on a normal diet.

It will be seen that the distinction between coeliac and normal cases is a fairly clear-cut one. Finally the three ringed dots represent the figures obtained from three normal children when on normal (lower area) and subsequently when on low carbohydrate diets (upper area).

There seems great difficulty in reconciling these intravenous results with those obtained by BADENOCH and MORRIS after subcutaneous injection of a similar dose of insulin. In all probability absorption of the insulin in
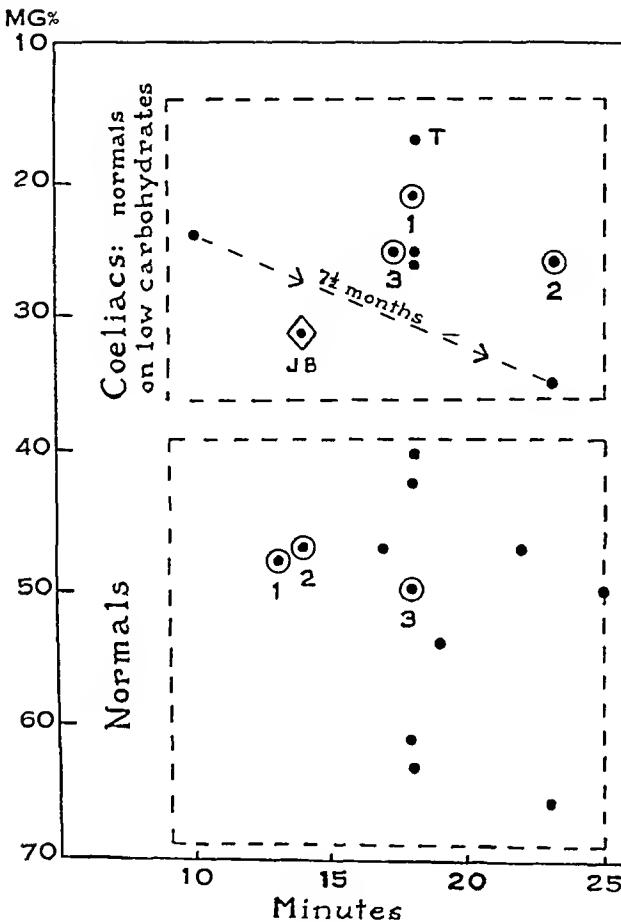
FIG. 9.—Maximal depressions obtained by giving four units of insulin intravenously. The plain dots represent in the upper rectangle coeliac cases, in the lower one normals. The ringed dots are three normal patients, below when on normal diet, above when on low carbohydrate diet.

The dot enclosed in a diamond represents a treated coeliac diagnosed at the age of 20 months, and now $12\frac{1}{2}$ years old on a normal diet.

their series has not been quite satisfactory, for among their non-coeliac cases one finds depressions as variable as 6·7, 15·1 and 29 mg. per cent., and it is, of course, well known that unabsorbed insulin is rapidly inactivated in the subcutaneous tissues.

THE EFFECT OF LIVER EXTRACTS ON CARBOHYDRATE METABOLISM.

There has been more and more clearly established in recent years, largely by the striking work of HIMSWORTH, the conception embodied in the second of the axioms which I presented earlier in these remarks—that " insulin sensitivity, and hence glucose tolerance, is due to the presence in the body of a third factor in the glucose-insulin reaction, now usually referred to as ' insulin-kinase.' "

For a great diversity of reasons, too diffuse to be discussed in this paper, it seemed probable that this substance, insulin-kinase, was produced, for the most part at least, in the liver, and pursuing this line it was decided to ascertain

the effect of an injectable extract of liver upon cases of impaired tolerance. Campolon was selected as being likely to contain any such principle as kinase, since it is prepared by simple expression methods without chemical alteration. Its injection intravenously was not advised by its makers, but the only untoward effect resembled a mild histamine reaction, the injection being immediately followed by a peripheral flush with a sense of great heat and some pulse accelera- tion, the whole passing off in about 2 minutes.

A number of patients were subjected to this experiment, not all suffering from coeliac disease but all showing impaired glucose tolerance. The causal conditions fall into two groups—those in which the impairment is mainly due to deficient absorption, namely coeliac disease, the closely related " chronic intestinal indigestion without steatorrhoea," and abdominal glandular tuber- culosis, on the one hand ; and on the other, disease of the liver itself, which has long been known to produce impairment of tolerance.

The mode of procedure was to give 2 c.c. of campolon in 20 c.c. of normal saline into a vein from 4 to 6 minutes before carrying out the ordinary intravenous tolerance test. In a number of cases further examinations were made after prolonged treatment with liquid extract of liver by mouth, usually half an ounce twice a day, which was found to produce a similar effect.

Abdominal glandular tuberculosis.

A. H., a boy of 9 years, was admitted to hospital with the history that for 6 weeks he had been getting very weak and wasting markedly. The appetite was poor. The weight was 42 lbs. After a week the temperature began to swing erratically up to 101° F., and there were the physical signs of a subacute peritonitis. The Mantoux reaction at a dilution of 1/1,000 was so markedly positive as to produce ulceration. After a total stay of some 4 months he improved considerably, the weight rising to 46¼ lbs. and he was sent to a convalescent home. There he remained quiescent for some 10 weeks before commencing to lose weight and to run a swinging temperature once more, with evening rises up to 100° F. After a month of this, his weight having fallen to 44 lbs., he was returned to hospital with a very poor prognosis. He was now quite unable to stand, even with assistance.

Fig. 10.—Intravenous glucose tolerance curves in a case of abdominal glandular tuberculosis. The upper curve represents the state before treatment with liver and again after withholding it ; the lower curve represents the effect of campolon intravenously and of liquid extract of liver by mouth.
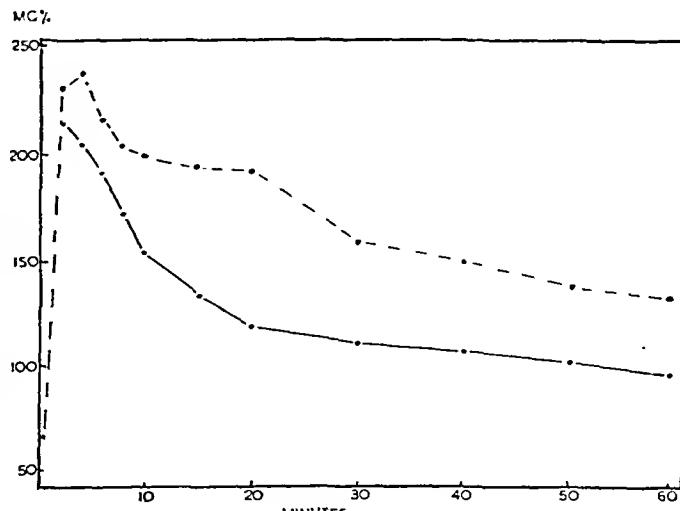
An oral glucose tolerance test gave the following figures (30 grammes) :

	Fasting	20	40	60	90	120	150 minutes.
Mg. per cent.	64	93	129	136	111	108	97

An intravenous test showed a gross impairment of tolerance (Fig. 10 upper curve). After an interval of 10 days, a further intravenous test was carried out, with an injection of 2 c.c. of campolon in 30 c.c. of normal saline 10 minutes previously. The result is represented by the lower curve in Fig. 10. In fact the tolerance had now been improved so that the intravenous glucose tolerance curve assumed a level which may be regarded as normal. The patient was now left without special treatment for 4 days, and an ordinary intravenous test carried out. The result was almost identical with that of the first test.

One fluid ounce of liquid liver extract (B.P.) was given daily for 2 weeks, and the intravenous test repeated. Apart from a small rise of 15 mg. per cent. in the fasting level, this curve was almost identical with that following the injection of campolon. The liver treatment was carried on uninterruptedly, and 4 months later a precisely similar curve was obtained, the fasting level having risen, however, to 99 mg. per cent.

The weight, which had fallen to 42 lbs. when liver therapy was commenced, rose in the following period of 11 weeks to 53½ lbs. Although there has been only a slight further gain in weight, the boy's general condition is excellent, and he is up and about and attending school.

Chronic intestinal indigestion.

M. P., a girl of 4 years, was admitted to hospital with the history that she had been a " feeding problem " since infancy, having spent a long period in an institution on this account. The abdomen had always been prominent, though the buttocks were normal. The appetite was always poor. The weight was 27 lbs. only.

The faeces were found to contain only 1·6 grammes of fat daily.

An oral glucose tolerance test (30 grammes) gave a curve of the flat type showing :

	Fasting	20	40	60	90	120	150 minutes.
Mg. per cent.	61	80	101	127	92	84	78

An intravenous test gave a high curve (Fig. 11, highest curve).

Ten days later this test was repeated 6 minutes after the intravenous administration of 2 c.c. of campolon. The curve obtained is represented by the dotted curve in Fig. 11.
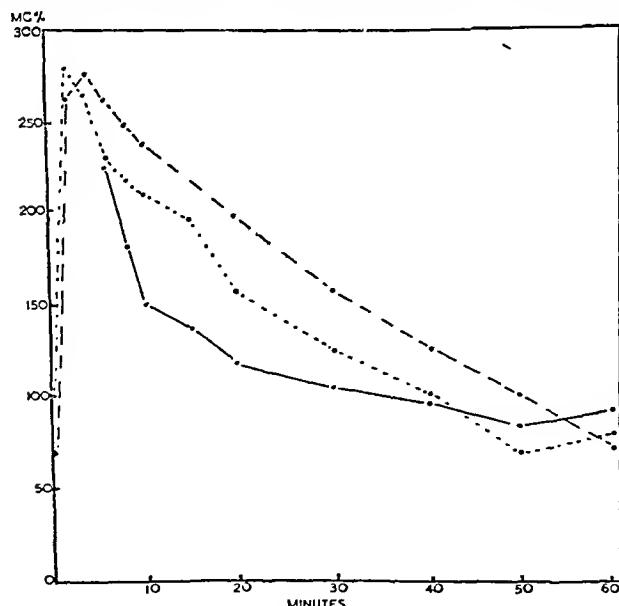
FIG. 11.—Intravenous glucose tolerance curves in a case of chronic intestinal indigestion without steatorrhoea, before treatment (long-dashed line), after campolon (short-dashed line), and after prolonged oral liver therapy (solid line).

After this test the patient was put upon a diet qualitatively low in carbohydrate and high in protein, and fat. At first there was difficulty in getting her to take it, but this notwithstanding she gained 4 lbs. in the ensuing 8 weeks. She was then transferred to a convalescent hospital and, while the diet was maintained, treatment by ½ fl. oz. of liquid extract of liver (B.P.) was commenced. The weight rose from 31 lbs. to 36¾ lbs. in the following 6 weeks and the child looked the picture of health. In demeanour too she had changed from silent, easily tearful depression to merry, playful activity. Her progress was then interrupted by a sharp attack of follicular tonsillitis, which entailed a loss of 2 lbs. in weight. A week after the temperature became normal, however, she had begun to regain this loss, and a final intravenous test was done. The resulting curve is shown by a solid line in Fig. 11. It represents normal tolerance.

Coeliac disease.

R. H., a boy of 18 months, was admitted to hospital with the history that 2 months previously he had begun to have frequent loose, pale stools, and to vomit occasionally. He had become listless and lost weight and the abdomen had increased perceptibly in size. Analysis of the stools over 3 days showed a daily output of 2·5 grammes of fat, of which one third was neutral fat. An oral glucose tolerance test was done (26 grammes only of the 30 gramme dose were taken), with the following flat result.

Fasting	20	40	60	90	120	150 minutes.
Mg. per cent. 80	95	111	111	103	90	82

An intravenous test showed (Fig. 12, solid line) the usual impairment of tolerance.

He was then given ½ fl. oz. of liquid extract of liver (B.P.) twice daily for 14 days, and the tests were repeated. The oral result (25 grammes) was :

Fasting	20	40	60	90	120	150 minutes.
Mg. per cent. 91	102	117	110	100	86	76

This curve was almost identical with the previous one except for the higher fasting level.

The intravenous result was markedly improved (dotted line, Fig. 12) the level at 60 minutes being 53 mg. per cent. lower than before.
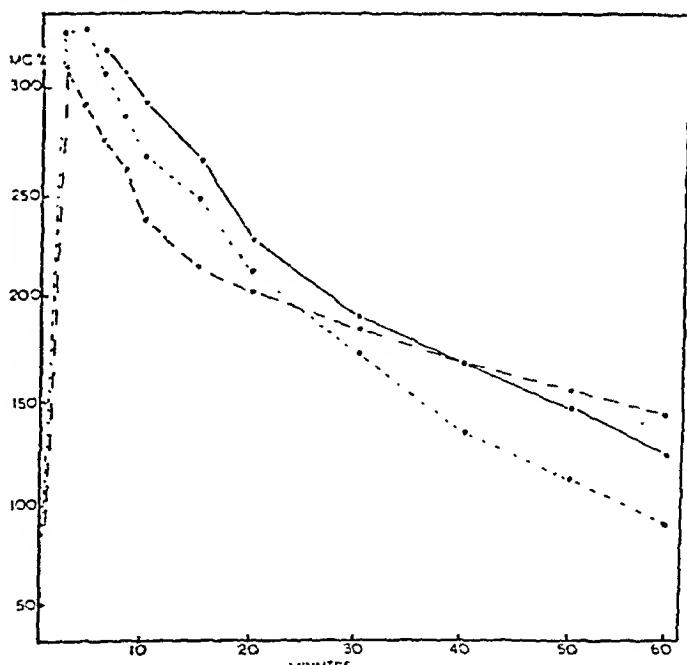
FIG. 12.—Intravenous glucose tolerance curves in a case of coeliac disease, before treatment (solid line), after liver extract by mouth (dotted line) and after cessation of treatment (dashed line).

The administration of liver was now stopped for 2 weeks and the curves repeated. The oral one was practically unaltered, while the intravenous one had reverted to almost its previous level (dashed line Fig. 12).

The clinical improvement in this case was difficult to assess, as he shortly contracted a series of infectious illnesses ; these, however, have been borne remarkably well.

Further reference must now be made to M.K., the case of coeliac disease in which continuous intravenous glucose therapy was given on two occasions. Some $3\frac{1}{2}$ months after the second of these occasions, her condition was far from satisfactory, there being a good deal of oedema and the weight being stationary. It was decided to give 2 c.c. of campolon three times weekly. Over the ensuing 5 months there has been a striking clinical improvement, with an increase of weight from $16\frac{1}{2}$ to 30 lbs. despite the loss of most of the oedema, and a child who was notoriously moody has become contented and playful, and has regained the use of her legs after being off them for about 9 months.

A recent intravenous glucose tolerance test showed a result practically overlying that obtained by the continuous administration of glucose 9 months previously (Fig. 7).

The blood in this case has never shown more than a mild secondary anaemia.

HEPATIC DISEASE.

Turning now to the hepatic cases, for access to all of which I am indebted to the Physicians of the Birmingham General Hospital, the first is an adolescent cirrhosis of unknown aetiology. The upper curve in Fig. 13 represents the original state of tolerance, the lower the result of 15 days on $\frac{1}{2}$-ounce of liver extract by mouth thrice daily.

The second is a case of diabetes mellitus with cirrhosis, probably due to haemochromatosis, which I was able to examine before any treatment had been
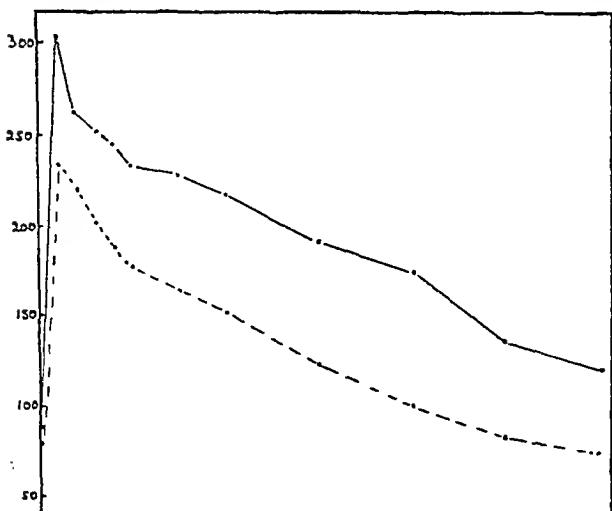
Fig. 13.—Intravenous glucose tolerance curves from a case of adolescent cirrhosis before treatment (upper curve) and after liquid extract of liver by mouth (lower curve).

given. An insulin depression curve (4 units) showed a depression of only 16 mg. per cent. on a fasting level of 270 mg. (Fig. 14, solid line). Subsequently this test was repeated, preceded by the injection of 2 c.c. of campolon in saline.
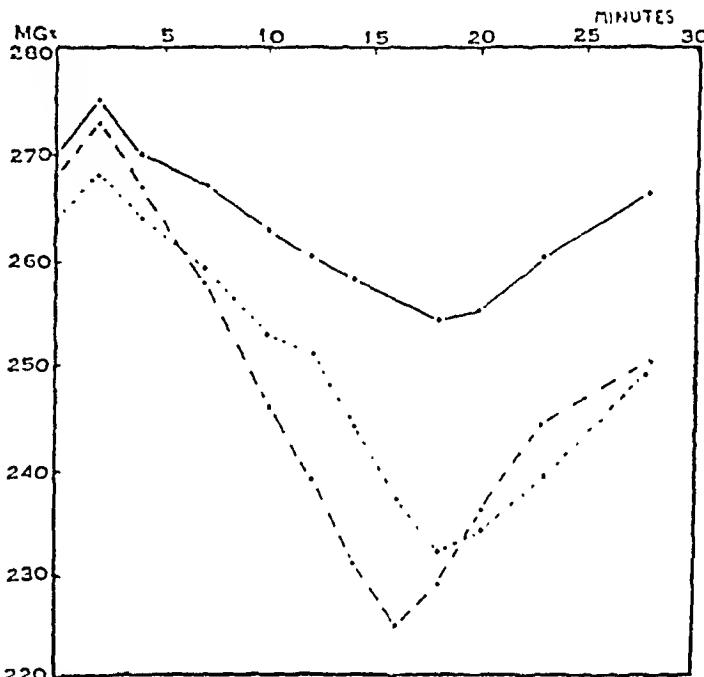
Fig. 14.—Insulin depression curves from a case of diabetes mellitus with cirrhosis of the liver, probably due to haemochromatosis, before treatment (solid line), after campolon (long-dashed line) and after liquid extract of liver by mouth (short-dashed line).

The resulting curve (dashed line) showed a depression of 43 mg. per cent. One ounce of liver extract was then given by mouth twice a day for 17 days, and the test repeated. The curve now obtained (dotted line) was generally similar to that after campolon, with a depression of 32 mg. per cent.

THE EFFECT OF CAMPOLON ALONE.

It will perhaps be asked what is the effect of campolon alone on the blood sugar ? Does it potentiate insulin or merely mimic it ? Fig. 15 shows the
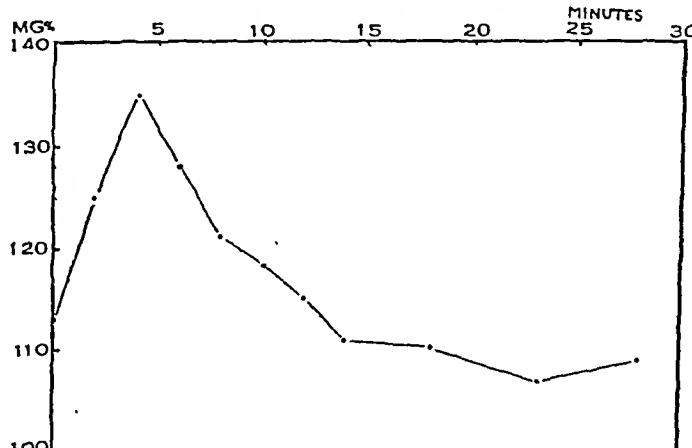
Fig. 15.—Variation of blood-sugar after the injection of campolon alone intravenously.

alteration in blood sugar values after giving 2 c.c. of campolon in 20 c.c. of normal saline intravenously to a normal adult. It will be seen that there is a rise of 22 mg. per cent. corresponding pretty closely with the period of flushing, followed by a simple restitution, with a fall of 6 mg. per cent. below the fasting level such as one expects after a period of hyperglycaemia.

PURIFIED P.A.F. PREPARATIONS NOT EFFECTIVE.

There is but one more point with which I have to deal. Is the tolerance-improving factor in liver extract identical with the anti-anaemic principle ? At the time that this was engaging one's attention Dakin and West's fraction was marketed as anahaemin (B.D.H.) after thorough assay of its anti-anaemic potency : and this offered the opportunity of comparing the action of a purified preparation with that of the total extracts such as campolon, or those made for oral administration.

A case of alcoholic cirrhosis which Dr. ERNEST BULMER had been treating for some days with injections of campolon for a macrocytic anaemia was by his kind permission subjected to an intravenous glucose tolerance test. While having campolon the lower curve in Fig. 16 was obtained. An equal dosage of anahaemin was instituted for 5 days, and the tolerance test repeated. The curve had now risen to the height and form one expects in gross cirrhosis (upper curve, Fig. 16).
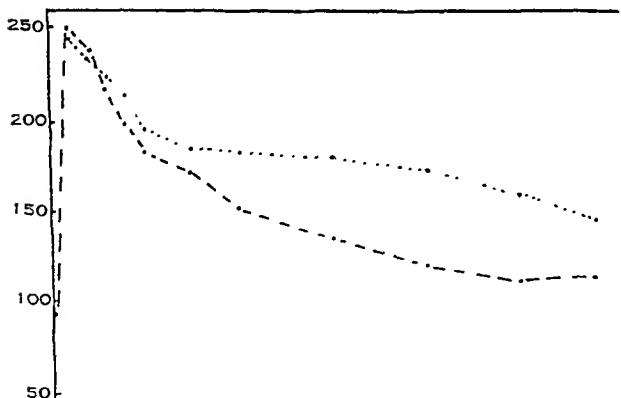
FIG. 16.—Intravenous glucose tolerance curves from a case of alcoholic cirrhosis after campolon treatment (lower) and after anahaemin (upper).

The reverse order of procedure was adopted in several cases of absorptive disease, and of these I present a typical one—a case of chronic intestinal indigestion. Before treatment an intravenous glucose tolerance test gave the upper curve in Fig. 17. When 2 c.c. of anahaemin was given intravenously before a further test a very slight improvement was noted (dotted line, Fig. 17) ; whereas under similar circumstances the administration of 2 c.c. of campolon led to a curve (dashed line, Fig. 17) approaching normal.

FIG. 17.—Intravenous glucose tolerance curves from a case of chronic intestinal indigestion before treatment (solid line), after anahaemin (dotted line) and after campolon (dashed line).
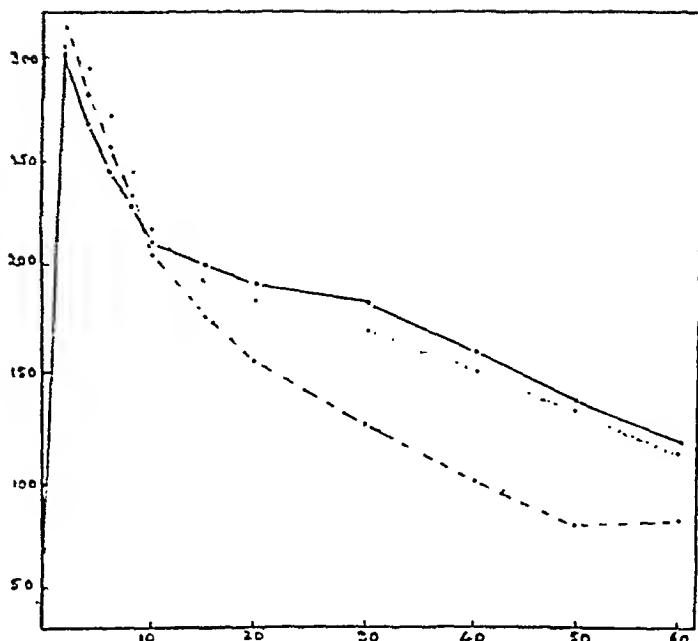
In conjunction with these observations it is most interesting to read some remarks contained in MILLER and RHOADS'S paper, published in 1934, on " The intensive liver extract therapy of sprue."

" The liver extract preparation found to be most effective is a relatively simple unconcentrated product. Evidence is at hand that a certain loss of activity results from too great refinement and concentration. Patients with sprue have failed to improve on intramuscular injection of a large amount of a highly refined and concentrated product, although a full remission has been effected by the use of a much smaller amount of material more simply prepared."

In conclusion, I think it is important to say that, although I have repeated the oral tests in cases treated by liver on several occasions, I have so far found no evidence whatever that absorption of carbohydrate is affected.

SUMMARY.

Firstly, evidence is advanced to show that there is defective absorption of carbohydrate in cases of coeliac disease, namely :—

(a) The association of flat oral with high intravenous glucose tolerance curves.

(b) The improvement of these intravenous curves towards normality by intravenous glucose therapy.

(c) The existence of a state of relative insensitivity to insulin comparable to that of a normal person on a low carbohydrate diet.

Secondly, it is shown that total liver extracts contain a factor, possibly identical with " insulin-kinase " capable of improving the glucose tolerance and sensitivity to insulin where it is impaired by the carbohydrate deprivation consequent upon defective absorption or by liver disease, sometimes to the great clinical benefit of the patient.   This factor does not seem to be identical with Castle's essential factor, and is more active in campolon than in anahaemin.

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DISCUSSION.

Professor L. G. Parsons : I have seen a certain number of cases (nine) of what I prefer to call " coeliac disease in adults," in two of which I know the condition started in childhood because they had been under my care sporadically in childhood, and I saw them later as examples of coeliac disease in adults. One of my patients developed the disease at 72 years of age, and another shortly after the operation of partial gastrectomy. Why a patient should develop the disease within a few days after partial gastrectomy I cannot say, and I have not met anybody who can explain it, perhaps Dr. FAIRLEY can. My experience has been chiefly with coeliac disease in childhood of which I have seen over one hundred instances. With regard to the question as to whether tropical sprue, non-tropical sprue (coeliac disease of adults), and coeliac disease in childhood are all the same disease : I would say that, although many symptoms are common to all three diseases and from a clinical standpoint I cannot see any difference between coeliac disease in childhood and coeliac disease in adult life, there are nevertheless certain differences between coeliac disease and sprue, particularly in relation to the blood pictures of the two conditions ; and, as Dr. FAIRLEY has pointed out, osteoporosis, an important symptom in coeliac disease, does not occur in tropical sprue. Again, except for the occasional occurrence in coeliac disease of megalocytic anaemia and slight glossitis I cannot see any real connection between pernicious anaemia, tropical macrocytic anaemia and coeliac disease ; further I think that the finding of Dr. Ross—that although the intravenous injection of campolon improves the impaired glucose tolerance in coeliac disease similar injections of anahaemin have no effect upon it—constitutes an insuperable objection to the suggestion that coeliac disease and pernicious anaemia are " conditional deficiencies caused by lack of specific reaction " between extrinsic and intrinsic factors. In this connection I have made two clinical observations which seem to me difficult to explain, but perhaps later speakers will be able to give an explanation : first, the development of megalocytic anaemia and symptoms of sub-acute combined degeneration of the cord in an adult with coeliac disease ; secondly, cure of megalocytic anaemia by the exhibition of ventriculin in a child suffering from coeliac disease, which, incidentally, cuts across the suggestion that the causation of sprue is absence of the extrinsic factor. This discussion is on the intestinal absorption in the steatorrhoeas and I would like to stress the fact that whatever be the cause of coeliac disease, its symptoms are those of a deficiency disease *par excellence*, for, in what deficiency disease is there such a permutation and combination of deficiency symptoms as osteoporosis, rickets, tetany, convulsions, skin manifestations, scurvy, beriberi, xerophthalmia, cataract, stunting of growth, dental hypoplasia, etc. ? Yet we have been told by Dr. FAIRLEY that there is no deficiency in the diet of sufferers from sprue ; indeed, the diet he puts on the screen would shame any suggestion of deficiency. I should have thought it was more than a sufficiency.

We have actually, therefore, an example of the well-known socialist slogan, " starvation in a land of plenty " ; starvation which is due, not to defective diet, but to defective absorption.

The study of coeliac disease presents three problems for some of which I cannot find an explanation, and I hope that either some of the succeeding speakers or one of the openers will solve them for me in his reply.

(1) If coeliac disease is a steatorrhoea only, why should scurvy, beriberi and deficiency anaemia occur ?   (2) Why do certain deficiencies occur in one case, and a different set in another ?   For instance, why should hypochromic anaemia, rickets, tetany and dental hypoplasia be relatively common, and xerophthalmia be excessively rare ?   (3) Why should the deficiency anaemia in coeliac disease be almost exclusively microcytic in character, whereas that in tropical sprue in constantly megalocytic ?

The answer to the first of these questions lies in the fact that although coeliac disease is called a steatorrhoea and fatty stools are its most striking feature, yet the conception of the disease as simply a steatorrhoea is an incomplete one ; rather it must be regarded as a condition in which there is impaired general intestinal absorption. British paediatricians, having been led away by the persuasive eloquence of my friend, Dr. REGINALD MILLER, have held that the essential deficiency in coeliac disease is mal-absorption of fat, and that the essential thing in its treatment is the strict limitation of fats in the dietary. MILLER believes that carbohydrate indigestion is a secondary symptom which only occurs during an attack of diarrhoea or a complicating entero-colitis, although he admits that excess of carbohydrate may make the stool bubbly, an observation with which everyone will agree and which was made by GEE, in his description of the stool as " yeasty and frothy, an appearance probably due to fermentation." In America, as long ago as 1908, HERTER held the contrary view, namely, that fats were better tolerated than carbohydrates, and in 1909, HEUBNER in Germany emphasised the importance in treatment of a diet which was low both in carbohydrates and fats. The case for the important part played by defective carbohydrate absorption in coeliac disease was, however, most strongly stressed by HOWLAND. HOWLAND wrote, in 1921, " From clinical experience it has been shown that of all the elements in the food, carbohydrate is the one which must be excluded rigorously ; that with this greatly reduced, the other elements are almost always well digested, even though the absorption of fat may not be so satisfactory as in health." For a long time I have been convinced that British paediatricians have underestimated the importance of defective carbohydrate absorption, just as, I think, HOWLAND under-rated the importance of defective fat absorption and the results which Dr. Ross has described are convincing proof that lack of absorption of carbohydrate is an essential thing in the symptomatology of coeliac disease ; indeed, that there is in coeliac disease a glycorrhoea as well as steatorrhoea. There is, however, one point in this carbohydrate metabolism which is difficult to explain, namely,

that oral administration of a mixture of equal parts of levulose and glucose produces a blood sugar curve similar to that obtained in the normal person. This fact is said to furnish the explanation of the effectiveness of ripe bananas in the treatment of coeliac disease because the sugar in it is invert sugar. Apropos of which form of treatment, a distinguished paediatrician in America has said that since the original description by Haas most of the American papers on coeliac disease have read like advertisements for the United Fruit Company. Is it possible that this different response to levulose indicates a selective activity in the absorption of carbohydrates?

If we study the absorption in coeliac disease, there seems clear clinical evidence of defective absorption of Vitamins A, C, D, the B complex, calcium, phosphorus, iron (perhaps copper) and the haematinic factor (or possibly lack of its formation). Protein seems to be the only thing left, and even it is badly absorbed during the diarrhoeal periods.

Another thing in respect of which I should like help in the discussion is, what term we shall apply to a condition in which there is steatorrhoea, glycorrhoea and azotorrhoea. The only term for this flux of foodstuffs seems to be " diarrhoea " but here we are faced with the difficulty that many coeliac children do not have diarrhoea !

I do not know any answer to my second problem—why certain deficiencies occur in one case, and why others occur in another—unless selective absorption is a possible explanation, that is to say, the assumption that absorption or lack of absorption varies from time to time. In rickets growth is an important factor and this may be an explanation of why children suffering from coeliac disease appear to stand shortage of Vitamin A better than of Vitamin D ; also it is possible that hypochromic microcytic anaemia may be related to the presence of achlorhydria, since both of these are common findings in coeliac disease.

Then there is, thirdly, the question of the anaemias. Hypochromic microcytic anaemia is very characteristic of coeliac disease, thus, in a series of thirty-five cases, twenty-one showed this type of anaemia, and three others passed, under treatment, from a megalocytic to a microcytic anaemia. It is interesting to enquire why megalocytic anaemia should ever occur in coeliac disease at all ? My colleagues and I have thought that megalocytic anaemia only occurs in old standing untreated cases of coeliac disease, and, except for those cases in which results of treatment have shown that the intrinsic factor is lacking, we came to the conclusion that the condition was due either to defective absorption of the haematinic factor which was more marked in diarrhoeal periods, or alternatively that it was a stage in the development of an aplastic anaemia.

Until to-night I was under the impression that Dr. FAIRLEY held that in tropical sprue achylia gastrica was associated with megalocytic anaemia and that therein lay an explanation of the megalocytic character of the anaemia ; that the difference between anaemia in coeliac disease and sprue might be due to the fact that whereas achlorhydria gastrica was common in coeliac disease,

achylic gastrica never occurred ; and that, just as in WITT's anaemia the achlor-hydria was associated with a microcytosis, so also the anaemia of coeliac disease was a mycrocytic one.   To-night, however, Dr. FAIRLEY's figures seem to show that achylic gastrica is not as common in tropical sprue as megalocytic anaemia is in that disease.

The form of erythroblastic anaemia, which can be cured by iron and which has been described by BENNETT, HUNTER and VAUGHAN, has not been seen in our series of cases ; but we have seen a condition in which there was a micro-cytosis without anaemia, the cell count being normal, and the cells in the blood film were deeply stained.   This occurred in three children, and has been described by my colleague, Dr. SMALLWOOD, who thinks the probable explanation of the condition is that the cells are thicker and more globular than normal, being similar to those found in acholuric jaundice, although these children did not show evidence of that disease.

Dr. H. P. Himsworth: I agree with the evidence brought forward by Dr. FAIRLEY and Dr. ROSS that I was wrong in my suggestion that the low blood sugar curve in steatorrhoea was the result of such cases absorbing a predominantly carbohydrate diet.

Having made one suggestion which has turned out to be wrong, I naturally feel hesitant about making another ; therefore, may I make an insinuation ? Dr. FAIRLEY brought forward convincing evidence that in these cases calcium is not absorbed from the gut.   JOHNSON, working in the United States, has shown that glucose absorption from the gut is inhibited when calcium gluconate is present.   May there be a connection between the deficient absorption of glucose in steatorrhoea and the presence of an excess of unabsorbed calcium in the gut ?

With regard to Professor PARSONS' question as to why the cases under discussion show an unusually small rise of blood sugar after oral glucose and·yet a normal rise of blood sugar after oral laevulose, I would suggest that an explanation is possible on the grounds of selective absorption.   Verzar has shown that whilst the absorption of glucose is a process invoking active work by the intestinal mucous membrane, the absorption of many other sugars is a simple physical process comparable to the diffusion of electrolytes through semi-permeable membranes.   As far as I know, it has not yet been decided how laevulose is absorbed, but if it should behave like a simple electrolyte, then the discrepancy between its effect and the effect of glucose on the blood sugar may be resolved.

I am greatly interested in the work of Dr. ROSS on the effect of campolon on the ability of the tissues to dispose of the carbohydrate.   I have investigated the effects produced by many liver extracts in the attempt to find a substance which had the actions I postulated for the hypothetical "insulin-kinase," but so far I have had no success.   With regard to the oral administration of liver,

I have given ½ lb. of raw liver daily for 10 days to a normal man on a low carbohydrate diet without producing the slightest effect on his sugar tolerance curve. I should like to know whether Dr. Ross has tried the effect of campolon in healthy people in reducing the high curve of a low carbohydrate diet to the low curve of a high carbohydrate diet.

Dr. Janet Vaughan : I use the term idiopathic steatorrhoea to describe that condition in which an excess of fat in the split form is excreted in the faeces by patients who have never been in the tropics. I do not include steatorrhoea due to organic lesions of the gastro-intestinal canal or to disorders of the pancreas. The relation of this form of steatorrhoea to tropi.al sprue and coeliac disease is controversial but not for discussion here. The symptomatology is varied, dependent on disturbances of (1) fat metabolism, (2) calcium metabolism, (3) carbohydrate metabolism, (4) haemopoiesis, and (5) probably protein metabolism. We suggested in 1932 that the varied clinical picture was dependent upon a disturbance of gastro-intestinal function resulting in deficient production, absorption and utilization of essential factors. (BENNETT, HUNTER and VAUGHAN, 1932.) At the moment we have no satisfactory direct method of studying intestinal absorption. When a substance given by mouth produces no effect, but is effective on parenteral administration it is assumed that there is a failure of intestinal absorption. The factors involved are, however, extremely complicated as a study of idiopathic steatorrhoea and other conditions has shown. The term failure of intestinal absorption must be considered to cover a wide range of reactions occurring in the intestinal tract and not simply to imply an inability either functional or organic on the part of the intestinal mucosa to pass essential factors across its surface.

It is generally agreed that all cases of idiopathic steatorrhoea show an increased excretion of fat which is, however, adequately split. In a series of eighteen cases I found the faecal fat to vary from 34 to 67 per cent. of the total faeces with an average value of 51 per cent. MONCRIEFF and PAYNE (1928) believe this excess of fat is due to re-excretion into the intestine. THAYSEN (1936) and his colleague have produced experimental evidence suggesting that intestinal absorption is at fault. They found the level of the fasting serum lipoid was constantly lower in patients with steatorrhoea than in normal adults, while following the ingestion of olive oil it rose higher in the latter than in the former.

Signs and symptoms of hypocalcaemia are almost invariably present in idiopathic steatorrhoea. Various explanations have been advanced to explain this ; (1) that it is due to some disturbance of the parathyroid mechanism, (2) it is dependent upon failure to absorb Vitamin D, (3) it is due to failure to absorb calcium. In an estimation of the calcium balance of eight cases (BENNETT, HUNTER and VAUGHAN, 1932) it was found that in four cases the total calcium output was greater than in the controls, in two cases the total output

was normal and in the remaining two, below normal. The discrepancy in these figures is more apparent than real, since no allowance has been made for the weight of the patients. In all cases the calcium excretion in the faeces exceeded that in the urine. The probable explanation of these findings is that the unabsorbed fatty acid in the alimentary tract combines with calcium salts to form soap, thus fixing the calcium. In part, therefore, calcium fails to be absorbed not because of any abnormality in the intestinal mucosa but because it is bound by fat. It is an indirect failure of absorption. There is considerable evidence, too, from a study of bile fistula dogs (HAWKINS and WHIPPLE, 1935) that a deficiency of Vitamin D results in a failure of absorption of calcium, again an indirect interference. It is presumed that the deficiency of Vitamin D is associated with the failure of fat absorption since Vitamin D is usually taken in the form of fat.

THAYSEN (1932) first showed that characteristically in idiopathic steatorrhoea a flattened sugar curve was obtained after ingestion of 50 grammes of glucose. He states that this is due to a disturbance of blood sugar regulation since he obtained similar flat curves after administration of glucose intravenously. I have seen a flat sugar curve in fourteen out of sixteen cases in which it was examined. In some instances such a flat curve was found even after great improvement had taken place in the general condition. In coeliac disease of infants (BADENOCK and MORRIS, 1936) improvement in the general condition results in a normal sugar tolerance curve. Following the intravenous injection of 50 grammes of glucose in one patient, who had previously had a flat curve, we obtained a marked rise in blood sugar. (Fig. 1.) The shape of the curve was similar to that obtained in two normal controls but the maximum level was appreciably higher than in the controls, and there was a greater divergence between arterial and venous levels which was maintained throughout. In the normal at the end of 2 hours the venous blood was higher than the arterial blood. (Fig. 2.) The significance of these findings is being further investigated. They suggest a failure of absorption of sugar and possibly also some difference of utilization in the normal person and in the patient with steatorrhoea.

The blood picture in steatorrhoea may be normal, there may be (1) a hypochromic anaemia, (2) a megalocytic hyperchromic anaemia, (3) a mixed type of anaemia. The hypochromic anaemia is relieved by large doses of iron. It is assumed, since these patients have usually had an adequate diet, that intestinal absorption is at fault unless extremely large quantities are available. We have no direct evidence that this is true. It is important to remember that absence of a rise in haemoglobin as BROCK (1935) has shown does not necessarily mean that iron has not been absorbed. The megalocytic hyperchromic anaemia may in some cases at least be relieved by giving extrinsic factor only in the form of large doses of mannite (VAUGHAN and HUNTER, 1932). The response obtained differs in no way from that in Addisonian pernicious anaemia following liver extract. Presumably there is, therefore, no lack of power to secrete
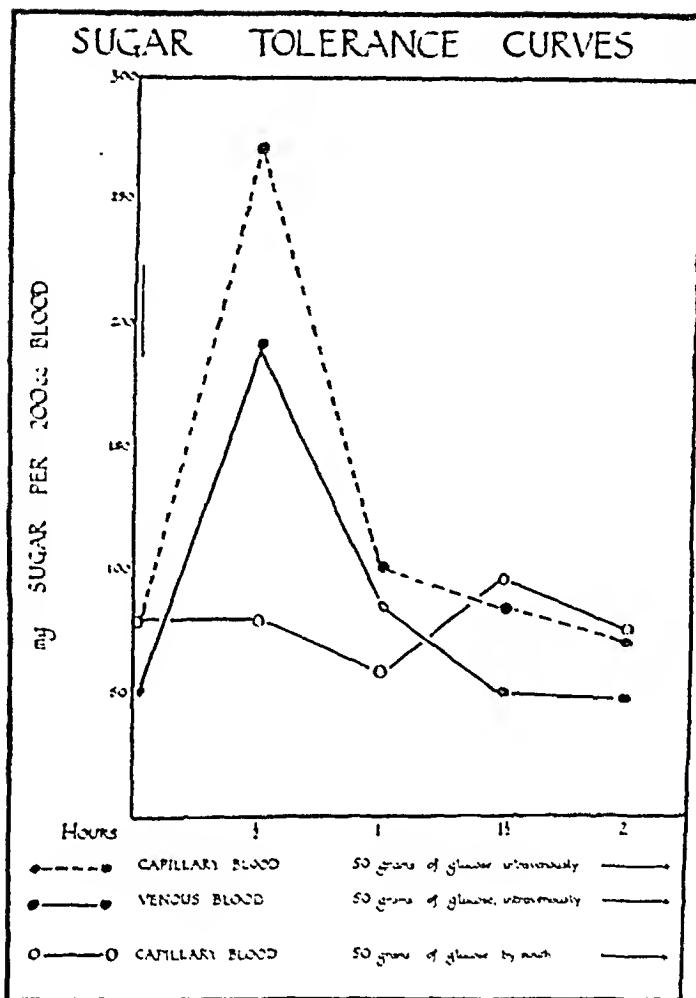
FIG. 1.—Sugar tolerance curves in a patient with idiopathic steatorrhoea.

intrinsic factor. The patients have previously taken a diet adequate in extrinsic factor content for normal people. One can only suggest that when increased amounts of extrinsic factor are given, increased amounts of P.A. factor are formed in the stomach, and that possibly large amounts are absorbed where small quantities are not.

The skin lesions in idiopathic steatorrhoea are improved when an adequate diet containing large quantities of Vitamin B is given. It is possible, however, that other factors such as Vitamin A are concerned (MACKAY, 1934). No controlled observations are available. There is no experimental evidence that intestinal absorption is at fault.

A study of idiopathic steatorrhoea suggests therefore that there is evidence of defective absorption of fat and of glucose in this condition. Defective absorption of calcium and of Vitamin D is second to that of fat. The disorders of haemo-poiesis are relieved by giving large quantities of haemopoietic factors, but we have

FIG. 2.—Sugar tolerance curve in a normal adult.

no evidence that there is defective absorption of these factors. The evidence at present available only justifies the statement that there is defective utilization.

The blood sugar curves following intravenous injection of glucose were studied in collaboration with Dr. E. J. KING.

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Dr. Reginald Miller: Prof. LEONARD PARSONS will not allow that fat mal-absorption is the essence of coeliac disease, and that the carbohydrate disturbance is secondary. He asks what proof there is of that. The proof of it, to my mind, is that you will see a great number of coeliac cases who are convalescent, and you will see a number of mild cases of the disease in which perhaps the amount of fat in the stools is up to sixty, but well soaped, in which there is no failure of carbohydrate absorption which one can detect. And I have noticed to-night that when speakers have been talking about carbohydrate they have been simply referring to sugar. But the starch in the food can be observed in the stools; and microscopical examination of many hundreds of samples of stools has shown me that in the types of coeliac case which I have mentioned starch is adequately absorbed.

There are one or two other points that I would like to mention. I am delighted that there seems to be general agreement to the effect that the faults in coeliac disease are due to mal-absorption; one after another has said so. This simplifies the problem very much. Dr. JANET VAUGHAN thinks that even the mal-absorption of fat is hypothetical rather than proved. This is admittedly a world of dubiety and uncertainty, but I must say, as far as I am concerned, that when a child weighing 15 lb. passes a stool weighing 2 lb., which contains in the dried state 80 per cent. of fat, that seems, to my uncultured mind, proof that fat-absorption is faulty; a simple-minded person like myself accepts that as good enough. May I suggest that it is possible that we could simplify the problem of steatorrhoea still further by assuming that the faults in absorption are all consequent upon the mal-absorption of fat, and that it is through mal-absorption of fat, especially in the form of fatty acid, that you get the wastage of the other materials, just as, in dosing a patient strongly with paraffin you get an excessive loss of all types of food. This single assumption would explain the loss of carbohydrate, vitamins, calcium and anti-anaemic factors. To this might be added some secondary factors reducing absorption: the marasmic condition of the patient, the starved condition of the intestine, and defective production of the digestive juices.

One other thing which is probably washed out in the excessive fat-loss is the tubercle bacillus. It is extraordinary how free the coeliac child keeps from abdominal tuberculosis, even though it has been a semi-invalid for years. We know that tubercle bacilli are absorbed from the intestine by the lacteals.

If we assume that the fat mal-absorption is the secret of the whole thing, that the wastage comes through that factor, there are only two problems left. One is the curious point, which is new to me, that in sprue, so it is said, there is no sign of osteoporosis. I think I am the only physician who has had a case of sprue in an English child, and that case I showed at this Society. I wish I had investigated the question of osteoporosis in that case. Many sprue patients are elderly and prosperous people who come quickly under treatment and are

FIG. 2.—Sugar tolerance curve in a normal adult.

no evidence that there is defective absorption of these factors. The evidence at present available only justifies the statement that there is defective utilization.

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rapidly improved, and I do not know how long it takes for osteoporosis to develop in steatorrhoea.

The other problem which remains unexplained is that in coeliac disease the anaemia is typically hypochromic. That may well have something to do with the age incidence and perhaps be produced by the intestinal toxaemia, rather than by the loss of anti-anaemic factors in the fat wastage.

Dr. Hamilton Fairley (in reply) : I did not mean to imply that osteoporosis never occurs in tropical sprue. In the investigation of a large enough series of cases, especially by special technique, it is probable that some evidence of osteoporosis would be found in chronic cases with persistent hypocalcaemia. In a limited number of cases, however, which we thought from the biochemical and clinical viewpoints might have presented osteoporosis, Dr. MATHER CORDINER was not able to demonstrate it, employing as controls people of a similar sex and age group. In the main the statement holds that hypocalcaemia is common and osteoporosis uncommon ; certainly it is not comparable in intensity or frequency with that observed in coeliac disease or idiopathic steatorrhoea.

Another point brought out clearly by the discussion is the fact that tropical sprue is altogether a milder breakdown than that encountered in coeliac disease and idiopathic steatorrhoea. Tropical sprue is readily curable and rarely manifests these other vitamin deficiencies which Professor PARSONS enumerated as common in coeliac disease. The flat glucose curves in our tropical cases change to normal within 2 or 3 months where the patient has clinically recovered. This is not the case, as Dr. ROSS has shown in coeliac disease, and Dr. VAUGHAN in idiopathic steatorrhoea. It is but another indication of the more intractable nature of the intest·nal breakdown in these diseases compared with tropical sprue.

Dr. Wallace Ross (in reply) : There are two points I would refer to. In reply to Professor PARSONS' question, I should like to associate myself with Dr. HIMSWORTH's " insinuation " ; I think his explanation of the better absorption of levulose is a reasonable one. In reply to Dr. REGINALD MILLER, we have one case of coeliac disease which has been under competent treatment 11 years, and is very well and has practically no steatorrhoea, but still shows a tendency to the type of curves which I have demonstrated. Also we have six other cases which are still suffering from coeliac disease but are improved, and these show a slighter improvement in the curves than they do clinically.

COMMUNICATIONS.

CLINICAL MANIFESTATIONS OF YELLOW FEVER IN THE WEST AFRICAN NATIVE AS OBSERVED DURING FOUR EXTENSIVE EPIDEMICS OF THE DISEASE IN THE GOLD COAST AND NIGERIA.*

BY

HENRY BEEUWKES, M D.

The history of yellow fever in West Africa, previous to the past decade, was concerned almost exclusively with the disease in Europeans ; cases among Africans were rarely observed, except when epidemics were present among the whites. During such epidemic visitations the number of cases diagnosed in Africans has varied considerably at different times. For example, no cases were recognized among the native population during the epidemic of 1925-26 in Lagos, Nigeria, or during an extensive outbreak in Senegal, in 1926 and 1927, which involved many towns and in the course of which approximately 350 cases were reported in Europeans, Syrians, and other persons of the white races. On the other hand, cases in Africans were reported or suspected in Lagos during the epidemic of 1913, in Accra in 1926 and 1927, and in the Belgian Bas Congo in 1927 and 1928. However, considering the enormous preponderance of black over white population in all these colonies, the number of cases of yellow fever recognized among Africans has been practically negligible. Extensive epidemics occurring exclusively among the natives have never been reported.

Detection of yellow fever in the African native and all problems connected with the disease in Africa are complicated by several factors : the number of trained physicians serving in the colonies where yellow fever is prevalent is exceedingly limited ; statistical reports of births and deaths, etc., are made only in a few of the larger centres ; burial of the dead in their homes is still a common practice ; the more primitive among the natives are fearful of the white man and have a tendency to resort to native medicine, witch doctors, the *ju-ju*, and what not, especially for the treatment of acute conditions. The majority of those applying for assistance from European physicians are persons

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with chronic illnesses who have failed to respond to native treatment.   Furthermore, the average case of yellow fever in the African, even during epidemics, is less severe and clear-cut than in the European.   Jaundice, one of the main symptoms of the disease, is more difficult to detect in persons of the black races ; and since an analysis of the urine is rarely made during routine examinations, albuminuria, another characteristic symptom of yellow fever, generally escapes detection.

These factors may explain, at least in part, the great discrepancy between reported cases of yellow fever and the actual incidence of the disease as indicated by protection test studies BEEUWKES *et al.* (1930, 1934, 1934), as well as the facts that the disease is rarely recognized in endemic areas and that extensive epidemics have occurred in various localities without discovery by local authorities.   That such epidemics, however, can be detected and that the disease in the African native is amenable to diagnosis will be evident from the descriptions which will be given below of many clear-cut clinical cases of varying degrees of severity which were studied by members of the West African Yellow Fever Commission in co-operation with local medical staffs during several visitations of the disease which occurred in recent years in Nigeria and the Gold Coast.

A brief account of the epidemics and description of the towns in which they occurred, together with such facts as could be collected in connection with the epidemiology of the disease in each, will precede the clinical discussion of cases observed.*

DESCRIPTION OF EPIDEMICS.

Asamankese.

The first of the epidemics upon which this report is based, and incidentally the first large outbreak of yellow fever exclusively among natives ever observed in West Africa, occurred during the spring and summer of 1926 at Asamankese, Gold Coast.   This is an important cocoa-trading centre 51 miles north-west of Accra, with a population of approximately 5,000, made up exclusively of natives.   The area in which it is situated is one of the most densely settled sections of the Gold Coast, and the numerous towns which are scattered throughout this cocoa belt are all connected with one another and with Accra and other coastal towns by excellent roads over which large numbers of persons are constantly travelling both by motor and afoot.   The town is rather less primitive than some of the others in which epidemics were studied.   The people are fairly prosperous and decidedly more intelligent than the average African native, and their standards of living are somewhat higher.   The homes, however, are almost exclusively one-storey buildings of mud construction, without any sanitary conveniences.

* Some data in connection with these epidemics have already been given in previous reports covering protection test surveys (BEEUWKES *et al.*, 1930, 1934, 1934).

The water supply is derived mainly from shallow wells and from a small stream which passes through the town. In addition, rain water is collected and stored in large tanks and barrels, and before the epidemic these, together with numerous earthenware jars, petrol tins, etc., served as prolific breeding places for the vector of yellow fever. The house index of *Aëdes aegypti* breeding at the time the study of the epidemic was initiated was 80 per cent.

Asamankese is under native administration, and at the time of the outbreak no European government officials or traders were permanently stationed there, although the administrative and medical officers of the district made occasional visits to the town. Owing to political difficulties, there was for the time being no recognized local chief, and for this reason it is not definitely known when yellow fever first made its appearance in Asamankese. It was first diagnosed on 9th July, when the District Commissioner had become suspicious because of great numbers of deaths which were reported to be occurring among natives, and had ordered a cadaver to be sent to Nsawam for pathological examination. It is probable, however, that the epidemic was present in fulminating form as early as 24th May, 1926, and it would seem that the disease was introduced during that month.

Those in control of the town were not kindly disposed to Europeans and refused outside medical assistance when an investigation was first undertaken by a member of the Yellow Fever Commission and the Director of the Medical Research Institute at Accra. These officers were informed by the acting chief that the deity was scourging his people and must be propitiated. A *ju-ju* man from Togoland had been employed at heavy expense to save them, and the Chief accordingly refused to exhibit any of the cases which he admitted were occurring in large numbers, though a few of the sick were observed in spite of his efforts to conceal them. Plans were made at once for a thorough study of the epidemic by several members of the Commission, and when we returned to the town the entire picture had changed. The *ju-ju* man had lost a considerable amount of his prestige through the death of his assistant from the disease and through the persistence of the epidemic in spite of his efforts. He had been further discredited when the sister-in-law of the Chief died and his son contracted the disease. Opposition to outside medical help was correspondingly decreased. The members of the Commission gradually gained the confidence of the people. In order to secure co-operation we treated all diseases. We administered neosalvarsan to persons suffering from yaws, and the spectacular results of this treatment were a potent factor in eliminating native prejudice. These facts are mentioned only to illustrate the difficulties encountered in securing information concerning outbreaks of disease among the natives even in densely populated areas and near important centres, and to show how possible it is that even large epidemics existing among these people may escape the notice of the authorities.

As is generally the case with epidemics of yellow fever in West Africa,

the source of the infection in Asamankese could not be definitely traced. It was probably introduced from Nsawam, a town 29 miles south-east and on the direct road between Asamankese and Accra, where an outbreak occurred during the previous March and April and was responsible for the death of two Europeans. Several non-fatal cases in Africans were also brought to light by careful search and interrogation of the contacts.

Statistical data concerning the number of cases which occurred during the outbreak are not at hand, but the number of deaths estimated after questioning the natives and counting newly made graves in and about the three local cemeteries varied between 123 and 178. The mortality among patients observed was approximately 13 per cent., and it was accordingly estimated that the total number of cases was over 1,000, or roughly 25 per cent. of the entire population. It was felt at the time that this estimate was conservative ; and the view was later confirmed after the development of the yellow fever protection test, when 40 per cent. of the sera of children of the town were found to show protective properties. One may assume, therefore, that many mild cases were constantly occurring unobserved and that the actual mortality rate must have been considerably lower than the figure (13 per cent.) based upon the number of cases studied.

It is interesting to note in connection with the epidemic in Asamankese that clear-cut cases of the disease were by no means limited to the adult population. Several severe as well as mild cases among children are included in the series reported below, and two babes in arms were seen who were definitely suffering from the disease. It was later ascertained, through interrogation of school children, that among sixty at one school, thirty had had a febrile disease which was probably yellow fever; while among 144 at the Scottish Mission School, four are known to have died of yellow fever, though many had left the town at the beginning of the outbreak.

One of the most striking features in connection with the epidemic is the fact that though conditions throughout this region would seem favourable for the dissemination of communicable diseases, careful search in many adjoining and more distant villages failed to bring to light any evidence of the existence of a similar recent infection in any of them.

Though the epidemic was on the wane when the study was initiated, a total of sixty-one cases, among the large number of persons suffering from various and sundry conditions treated, were diagnosed as probably yellow fever. In some of these the symptoms were very mild or the cases were complicated by tuberculosis or schistosomiasis, which tended to render the diagnosis less certain, while a few were seen too late during the course of the illness to permit of securing detailed data. Fifty definite cases were included in the series, however, and among these eight were fatal, fourteen were classified as severe, and an equal number were included in the categories of moderately severe and mild.

Suhum.

Following the epidemic at Asamankese, which terminated in September, 1926, there appeared a similar but less extensive visitation at Suhum, another town in the cocoa belt of the Gold Coast, situated 42 miles north of Accra, near Nsawam, and approximately 50 miles by road from Asamankese.

The conditions there which may have a bearing upon the epidemiology of yellow fever are in general similar to those prevailing in Asamankese. The community is in direct communication with the more important towns of the belt, and during the cocoa season, from September to mid-April, is an extremely active commercial centre through which large numbers of lorries and other vehicles pass daily en route north and south. The population had more than doubled during the 6 years preceding the epidemic and at the time of the outbreak was over 2,500, made up exclusively of natives and a few Syrian families. The inhabitants are relatively intelligent and well nourished, and their financial condition previous to the depression was satisfactory, as indicated by the fact that the expenditure *per caput* was said to have been 50 dollars annually. Nevertheless, their houses in general are small, of mud construction, and without sanitary conveniences.

For the water supply, reliance is placed partly upon the abundant rainfall which is collected in unscreened barrels, clay pots, and tins, but mainly upon several small streams which pass through the town. In spite of the fact that a sanitary squad composed of one headman and four labourers was stationed in Suhum, the index of *A. aegypti* breeding there, as in Asamankese, was exceedingly high prior to the outbreak—not less than 80 per cent.—and conditions in general were favourable for the propagation of the vector of yellow fever.

The sequence of events leading up to the discovery of yellow fever in Suhum was as follows: about 15th January, 1927, the sanitary headman reported an unusual number of deaths; on the 27th, Dr. MACKAY of the Colonial Medical Service and Dr. WALCOTT visited the town and saw a patient with symptomatology suggestive of yellow fever; three days later, Dr. MACKAY performed an autopsy upon a cadaver which was brought to Kibbi, and the presence of the disease was confirmed by gross and microscopic pathological findings.

Yellow fever was recognized in numerous places in the southern portion of the Gold Coast during 1926 and 1927, but its spread from point to point could not be traced, and it is not definitely known from whence the infection was introduced into Suhum. The fact, however, that epidemics had recently been present in Nsawam and Asamankese, which are in such close communication with this town, is suggestive.

Though no estimate could be made of the total number of cases in Suhum, it is probable that the percentage of persons affected was much lower than in Asamankese. The disease appears to have been promptly recognized in Suhum, and active and effective sanitary measures were instituted by the Government

without delay. These steps reduced the incidence of *Aëdes* breeding from 80 per cent. to approximately 5 per cent. within a month, and coincident with this, infection disappeared.

The total number of cases observed by members of the Commission and included in the study was twenty-five, of which five were fatal, eight severe, six moderately severe and six mild.

Larteh.

The third epidemic of the series was brought to light on 15th May, 1927, at Larteh, also in the Gold Coast and 35 miles north-east of Accra. The town is situated at an elevation of approximately 1,000 feet, upon the crest of a narrow sloping ridge along which it extends for about a mile. It is divided for administrative purposes into two parts called Upper Larteh and Lower Larteh, each of which is under a separate chief.

In contrast with the places already described, Larteh is a residential rather than a commercial centre. The majority of the inhabitants, however, all of whom are Africans, have farms at various distant points and are absent from the town during a portion of the cocoa season. Therefore, though the population in 1921 is officially given as over 4,000, the actual number of persons residing there at the beginning of the epidemic was probably not in excess of 2,500, divided about equally between the upper and the lower town. As many of the farms are situated near Suhum and at other places where yellow fever was previously present, it seems quite probable that the infection was introduced into Larteh by persons temporarily returning to their homes from the field.

The inhabitants are relatively poor, and the houses are generally small, unsanitated, one-storey structures of mud or stone. The water supply is derived mainly from a spring near the lower end of the town, and in order to avoid heavy porterage much water is stored during the rainy season, especially in the upper part of the town. Storage is generally in unscreened barrels, tins and other receptacles. The house index of *Aëdes* breeding in Upper Larteh on 15th May was almost 100 per cent., and all infected containers were found teeming with larvae. In contrast with these findings, an inspection made a few days later in Lower Larteh revealed an index of only 30 per cent., with intensity of breeding comparatively low.

Although the epidemic was not brought to the attention of the authorities until 15th May, it is certain that cases had been occurring in Larteh for at least some weeks previous to that time. It was ascertained from the Chief, who kept an unofficial and incomplete record, that there had been sixty-eight deaths in the upper town alone between 1st January and 15th May, and after interrogating relatives and friends of the deceased it was concluded that yellow fever was responsible for at least twenty-six of these and that the infection had probably been introduced during the month of February.

Anti-mosquito work was instituted immediately upon the discovery of

the epidemic, and by the middle of June the house index of breeding had been reduced to 5 per cent. The last case was seen on 6th June.

The actual number of cases observed after the outbreak was discovered was thirty-five ; and five, or 14·3 per cent. of these were fatal. Of the thirty-five cases, thirty-three occurred in the upper town, while the remaining two, which were the last observed, were in Lower Lartch but in close proximity to the administrative boundary.

Although accurate data on the epidemic were not available, it was estimated that the cases totalled over 200 and that the deaths were in excess of thirty. The disease was first introduced into the upper limits of the upper town and slowly and gradually extended downward, but it had made no real headway in Lower Lartch when sanitary measures were introduced and rapidly eliminated the infection. That it did not gain a foothold in the lower town may be explained on the basis of the relatively low house index of *Aëdes* breeding and the small number of these mosquitoes present, as well as the fact that the residents knew of the existence of the epidemic and avoided the infected section.

A protection test was later carried out upon the sera of twenty-five children from both upper and lower towns. Thirty per cent. of the former were positive, while no specimen from Lower Lartch showed protective properties. The protection test figures in the upper town suggest, as in Asamankese, that considerable numbers of mild unobserved cases must have occurred and that the actual mortality rate was under 10 per cent.

Of thirty-five cases studied in Lartch and included in the clinical report below, five were fatal, ten severe, nine moderately severe, and eleven mild. Though numerous cases both in Europeans and natives were seen during other minor epidemics in the Gold Coast, they are not included in the clinical study, which refers only to epidemics which occurred exclusively among African natives.

Ife, Nigeria.

The last of the major epidemics included in this report, and the only extensive visitation of yellow fever ever observed exclusively among natives in Nigeria, occurred in 1928 at Ife, an ancient and exceedingly primitive city of approximately 35,000 inhabitants, lying 55 miles due east of Ibadan on the main highway connecting that town with Benin City. Situated in the densely populated region of south-western Nigeria and having large markets, it is an important place commercially and has considerable intercourse with other cities of this region. It derives its main distinction, however, from the fact that it was the first settlement of the Yoruba people in Southern Nigeria and is mythically considered by them as the place where man was created. It remains the seat of the Oni, or high priest, of all Yorubaland, who has great power and is constantly visited by individuals and deputations from the different sections under his influence. Nevertheless, it is less progressive than the majority of other Yoruba

cities and is without sanitation or water supply. The house index of *Aëdes aegypti* breeding there has always been exceedingly high.

The outbreak in Ife was discovered early in June, 1928, when it was learned from an officer of the Department of Education, stationed in Ibadan, that an epidemic disease associated with jaundice existed there. Upon investigation by the Medical Officer of Health of the district and a member of the Commission, several suspicious cases were found, and a study by members of the Commission was at once undertaken. The epidemic had undoubtedly been present for some time before it was recognized. In consequence of an earlier report, a brief investigation had been made by government medical officers several months before but without definite results, and from facts ascertained it seems probable that the disease had existed, unrecognized, since November, 1927.

No information could be secured as to the origin of the infection, nor was it possible to obtain sufficient data to permit an accurate estimate of the number of cases that had occurred. The extent of the epidemic can be judged, however, from the findings of protection test studies carried out later upon the sera of two groups with twenty-five children in each which gave respectively 68 and 72 per cent. of positive results. Eighteen cases were diagnosed as yellow fever during the month of June, and twelve of these are included in the clinical study below; of this number six are classified as severe, four as moderately severe and two as mild. A few fatal cases were seen, but the data concerning these were insufficient to permit of clinical analysis. From two of the patients who were seen early, strains of yellow fever virus were obtained by inoculating their blood into *Macacus rhesus* monkeys, and the diagnosis in all of the series was later confirmed by protection test studies.

One of the interesting features of the epidemic was the apparent persistence of the infection in this town in spite of the considerable sanitary work carried out. The study was discontinued in July owing to the necessity of carrying out investigations in other areas. Suggestive cases, however, were present during the remainder of that year and also in 1929, when *Aëdes aegypti* captured in the town were demonstrated to be infected, by inoculation into *M. rhesus*.

CLINICAL FINDINGS.

The clinical data obtained in our studies will be presented under the following headings :

I. Brief Case Histories (p. 70) illustrating the varying symptomatology in fatal, severe, and mild cases of yellow fever.

II. Percentage Tabulation of Symptoms (p. 75) giving, by days, the percentages of the mild and the more severe cases observed in Asamankese, Suhum, Larteh, and Ife, in which the commoner signs and symptoms of yellow fever were present.

III. Clinical Analysis (p. 78).

Before the clinical data are presented it must be mentioned that the conditions under which the studies were carried out were far from ideal. The towns in which epidemics occurred have no resident physicians and no hospitals, dispensaries, or other facilities for the care and treatment of the sick. It was in consequence necessary to examine all patients in their homes, where they were generally found lying upon the ground in dark corners of poorly ventilated rooms. When practicable, they were brought to the door or into the yard for observation, while those who could not be moved were examined with the aid of a flashlight, taper, or some other artificial light. Such examinations usually stimulated the interest and curiosity of the neighbours, who crowded in large numbers into the compound or even into the sick room, causing the greatest din and confusion.

History taking was time-consuming, as many of the patients had no knowledge of English and satisfactory interpreters were difficult to obtain. Furthermore, statements made by the African native must be accepted with some caution. As patients were frequently moved without warning from their homes to those of friends or relatives or even to distant villages, when a fatal termination of the illness was feared, it was sometimes impossible to locate them, and their case records are, therefore, incomplete. In spite of this, however, it is believed that the data covering even the subjective symptoms are reasonably accurate and comprehensive.

As far as possible, at least one visit was made daily to the home of each patient. Blood examinations for malaria or other parasites, as well as white blood cell and differential counts, were made, and urine analyses with albumin determinations were carried out from day to day. In addition, much culture work was done and many laboratory animals were inoculated. This work was made possible in the Gold Coast through the kindness of the Director of the Medical Research Institute at Accra, who placed the mobile laboratory of that institution at the disposal of the Commission, and at Ife, through the organization there of a small field laboratory.

The West African native has the greatest antipathy to any mutilation of the body after death, and consent for autopsy is accordingly very difficult to obtain. Every effort was made to induce the relatives to permit postmortem examination in all fatal cases observed, but it was generally impossible to secure their consent for even partial section. Coercion was not attempted for fear of losing the co-operation of the people and, therefore, opportunities for study. However, a sufficient number of autopsies were performed to establish the diagnosis in the towns studied on the Gold Coast ; and later, protection tests carried out with the sera of considerable numbers of patients gave uniformly positive results. In Ife, the diagnosis was established by infectivity as well as protection tests. (See page 73).

I.—Case Histories.

1. *A fulminating, rapidly fatal case.*

Kofi Ajuri, male, 50 years of age.

The patient is a native of Asamankese, and during the epidemic he has referred a number of cases to members of the Commission for examination and treatment. He has always enjoyed good health, and has not been subject to fevers. He states that he has never had an illness similar to that from which he is now suffering and has never before observed an epidemic of this kind.

Present Illness.

This began 29th July with headache involving the frontal region and extending to the occiput, and pain in the back, epigastrium, and hips, but not in the limbs generally. Photophobia was marked. The patient was first seen 1st August, when he had a severe shaking chill, but with no rigor at onset. He has complete anorexia and has vomited a colourless fluid at intervals yesterday and to-day. He has taken very little water. His urine is scanty.

Physical Examination.

The patient looks very sick; he is prostrated and evidently in pain. His temperature is 102·8°; his pulse is 68, and is weak and of low tension; his respiration is sighing and grunting.

The sclerae are moderately injected and show a definite mild icterus. The tongue has a heavy white coat over the dorsum; the edges are moist and moderately red. The breath is foul. Teeth and gums are negative. The heart and lungs are normal. There is marked epigastric tenderness and a tendency to vomiting on slight pressure over the upper abdomen. The liver is definitely enlarged and markedly tender. The spleen is not palpable.

The urine is clear, acid, and mildly bile-stained. There are over 10 grammes of albumin per litre; three-quarters of the specimen completely solidified upon acidifying and boiling.

2nd August.—The patient's temperature is 101°, his pulse rate 69. He has no headache. Pain in the back persists, and pain has developed in the legs. He complains of soreness in the epigastrium and has no desire for food, but he is not vomiting. He has been constipated for 3 days. Icterus has increased and the eyes are much injected. The gums are congested and swollen but not hemorrhagic. Prostration is very marked, and the prognosis is bad.

The urine is slightly turbid, amber, and acid. Secretion is reduced. There is an exceedingly heavy precipitate of albumin. Microscopic examination reveals a fair number of granular casts and a few leucocytes and red blood cells.

Blood examination shows no malarial or other parasites. The number of leucocytes is diminished. The differential count is : polymorphonuclears, 62 per cent.; lymphocytes, 30 per cent.; and mononuclears, 8 per cent.

3rd August.—The patient was moved to another section of the town but was later brought back to his home, where he died at noon. He was not seen by any member of the Commission, but his family and friends state that he vomited black material during the morning and passed into coma before death.

2. *An unusually prolonged fatal case.*

Yabua Nelson, an intelligent male, 16 years of age.

The patient is a native of Asamankese, who speaks English well. He attended school at Abitifi, but has been in Asamankese continuously for the past 20 days. Two previous cases of yellow fever have developed in his house. The first was that of Ebenezer Sintim, his cousin and a teacher in the local Wesleyan school, who became ill on 13th July, and is now convalescent but still deeply jaundiced. The second was in Baby Ya, 6 months of age, seen on 17th July with fever, slight vomiting, and moderate icterus which persists.

Present Illness.

This began 19th July with a slight chill, fever, severe frontal headache, and pain in the back. The patient was first seen on 22nd July, when headache had disappeared but pain in the back persisted. He has had marked anorexia since the beginning of the attack, but is not vomiting. He has some diarrhoea.

Physical Examination.

The patient is a fairly well developed boy, weak, but walking around. His temperature is 102·4°, his pulse rate 72. His sclerae are icteric. His tongue is almost clean, and his gums and teeth are normal. His heart and lungs are negative. His liver is moderately tender and enlarged. His spleen is not tender, but is palpable 2 cm. below the margin of the ribs.

Blood examination reveals no malarial or other parasites. There is slight leucocytosis. The differential count shows 70 per cent. of polymorphonuclears, 26 per cent. of lymphocytes, and 4 per cent. of mononuclears and transitionals. One nucleated red cell and a small number of stipple cells are seen.

23rd July.—The patient's temperature is 102°, his pulse rate 82. Sclerae are very yellow. The tongue is flabby, slightly coated, and moist. The gums are normal. The liver is enlarged and tender. The urine is amber, acid, and slightly cloudy, with a specific gravity of 1015. Esbach test shows 1 gramme of albumin per litre.

24th July.—The temperature is 104°, the pulse rate 81. The sclerae are more deeply jaundiced.

27th July.—The temperature is 103°; the pulse is 105 and markedly dicrotic. The patient states that he feels better but complains of some pain in the knees and legs. He shows marked prostration and weakness. He has considerable epigastric tenderness and vomits a little. Urine secretion is reduced. The specimen examined is thick, cloudy, green, and heavily laden with bile ; it solidified almost completely upon heating and the addition of acetic acid ; the precipitate forms a green pasty mass. A centrifugalized specimen shows masses of bile-stained detritus but no casts.

28th July.—The patient's temperature is 102·2°, his pulse rate 93. His condition is daily becoming aggravated. Jaundice is exceedingly deep and visible on all mucous membranes. The liver is tender and enlarged to 6 cm. below the costal margin.

29th July.—The temperature is 99·6°, the pulse rate 87. The patient is weak, toxic, and mentally dull.

30th July.—The temperature is 99·4°; the pulse rate 87. Anuria is beginning.

31st July.—The temperature is 98·4°; the pulse rate 87. There is profuse bleeding from the gums.

1st August.—The temperature is 98·8°; the pulse rate 84. Melena and parotitis have developed.

2nd August.—The patient has bleeding gums, bloody vomitus, melena, and muscular twitching. Coma set in, followed by death.

3. *A severe case with deep persistent jaundice and heavy albuminuria.*

Kwami Otchere, a male, 26 years of age.

The patient was born in Larteh and has lived there all his life. He has had no previous illness.

Present Illness.

This began 16th May with rigor, fever, headache, photophobia, pain in the back and chest, and mild diarrhoea. The patient is weak but is able to be up and about.

Physical Examination.

The patient's temperature is 103°, his pulse rate 93, and respiration 28. The eyes are congested but show no icterus. The tongue is typical of yellow fever in appearance : it is small, pointed, with red tip and edges and a moderate coat. The chest and the

abdominal viscera are normal. The liver and spleen are neither enlarged nor tender.

Blood examination shows no parasites. The haemoglobin is 80 per cent. Leucocytes are normal in number, with 61 per cent. polymorphonuclears, 24 per cent. lymphocytes, 10 per cent. mononuclears, and 5 per cent. eosinophiles.

17th May.—The temperature is 102·5° ; the pulse rate 94. Headache persists. There is marked pain in the spine, some prostration, and anorexia without vomiting. The patient is up and about. His eyes are congested, but he has no icterus. His gums are normal, and the condition of his tongue is unchanged. His chest and abdominal viscera are normal. His urine is clear, amber, and acid, with a light cloud of albumin and no casts.

18th May.—The temperature is 102·8° ; the pulse rate 72 ; respiration 32. Prostration has increased and the patient is lying down. He complains of insomnia, headache, pains in the spine, and some pain in the chest. Anorexia and vomiting are marked. The eyes are greatly congested and scleral icterus has developed. The condition of the mouth is unchanged. Epigastric tenderness is marked. The liver is enlarged and tender. Albumin is much increased, being equal to one-fifth of the total volume of urine boiled.

19th May.—The temperature is 100·4° ; the pulse rate 72. Headache has disappeared, but pain in the spine, anorexia, and vomiting persist. The eyes remain congested, and scleral icterus has somewhat increased. The condition of the mouth is unchanged. The epigastrium, liver, and spleen are very tender. Albuminuria is heavy, the Esbach test showing 5 grammes of albumin per litre of urine. There are a few granular casts.

20th May.—The patient's temperature is normal and his pulse rate 66. He is lying down and is very ill. Pain in the spine continues. Scleral icterus is now deep. The appearance of the tongue is still typical of yellow fever. Epigastric tenderness, with enlarged and tender liver, persists. The urine is alkaline and contains bile. The albumin content is 9 grammes per litre, approximately double that of the previous day. Many bile-stained casts are seen in a centrifugalized specimen of the urine.

21st May.—The patient's temperature is normal, and his condition is improving ; but prostration continues. Scleral icterus remains very deep. The appearance of the tongue is the same as on the previous day. Abdominal symptoms persist. Albuminuria is unchanged. A centrifugalized specimen of urine shows an occasional bile-stained granular cast.

Convalescence was fairly prompt. All symptoms had cleared up by the 14th day with the exception of jaundice, which persisted for some weeks.

4. *A severe case, showing heavy albumin but practically no jaundice.*

Abina Ababio, a female, 25 years of age, married and the mother of two children.

The patient was born in Asamankese and has always lived here. She claims that she has never been sick before.

Present Illness.

This began 2nd August with fever, severe headache, pains throughout the entire body, marked anorexia, but no vomiting. These symptoms persisted until the morning of 4th August, when the patient's condition was as follows :

Physical Examination.

Temperature 102·5° ; pulse rate 96. Eyes considerably congested, but showing no icterus. Tongue small and pointed, with typical red edges and tip, but uncoated ; teeth and gums negative. Heart showing no abnormality. Lungs presenting distinct rales at the right base posteriorly.

Blood examination reveals no malarial or other parasites. There is marked leucopenia. The differential count is : polymorphonuclears, 42 per cent. ; lymphocytes, 52 per cent. ; mononuclears and transitionals 6 per cent. The haemoglobin is 80 per cent.

The urine is turbid, straw coloured, acid. There is a considerable albumin deposit— 1½ grammes per litre by Esbach test. After boiling and the addition of acetic acid, a specimen was allowed to stand over night ; albumin then equalled one-sixth of the column

of urine boiled. A moderate number of small granular casts are seen in a centrifugalized specimen, but there is no pus or blood.

5th August.—The patient's temperature is 103°; her pulse rate 78. She has no headache, epigastric pain, or vomiting, but complains of moderate backache, pain in the legs, and soreness in the upper thorax, with slight cough and catarrh. The sclerae show no icterus. The tongue is small, with red edges and tip, and a slight white coat. The liver is slightly enlarged and palpable, but not tender. The spleen is neither palpable nor tender. There is some epigastric tenderness.

There is a heavy deposit of albumin in the urine equalling two-fifths of the column of urine boiled. Granular casts are numerous, but there is no pus or blood.

6th August.—The patient's condition is improved but she is prostrated. Her temperature is 101°, her pulse rate 81. She has a slight cough. The tongue is clear; the teeth and gums are negative. There is no epigastric, splenic, or hepatic tenderness, and no icterus. Albuminuria is very heavy, the Esbach test showing 3·5 grammes of albumin per litre of urine. Microscopical examination of the urine shows occasional granular casts.

7th August.—The patient's temperature is 103·2°, her pulse rate 90. She is weak and complains of backache, but has no other pains and no icterus. Her tongue, teeth, and gums are normal. There is no epigastric tenderness.

8th August.—The patient feels and appears better, but has slight pain in the back and in the front of the chest. Her temperature is 101·7°; her pulse rate 90. She is not vomiting, and has no icterus. The urine shows 20 grammes of albumin per litre by the Esbach test. A specimen almost completely solidified upon boiling. Microscopic examination reveals an enormous number of small granular casts, but no blood or pus.

9th August.—The patient is sitting up but looks ill. She still has severe headache, some cough, and soreness in the chest. Her temperature is 102·4°; her pulse rate 98.

10th August.—The patient shows marked improvement. She has no pain but is very weak. Her temperature is 99·4°, her pulse rate 69. The sclerae show a scarcely perceptible tinge of icterus. The albumin content of the urine is still exceedingly heavy; practically the entire contents of a tube solidified on boiling. Granular casts are fairly numerous.

11th August.—The patient's temperature is 102·3; her pulse rate 80.

The woman made a slow and uneventful recovery. Albumin gradually decreased and had practically disappeared by 17th August. A protection test carried out in 1933 with her serum, diluted 1 : 10, was positive.

5. *A moderately severe case of relatively short duration.*

Itagbosin, a male, 60 years of age.

This case is of particular interest, as it was among the first diagnosed as yellow fever at Ife, Nigeria, and one of three definite cases seen in the same home, the son and daughter-in-law of the patient being ill at approximately the same time. At least two other suspicious cases had previously occurred in this house.

The diagnosis was confirmed by injecting the blood of the patient into a *Macacus rhesus*. After six days the animal developed fever (104·2°), which continued for 2 days and reached a maximum of 105·2°. It died 2 days later, and both gross and microscopic pathological findings were typical of yellow fever. The strain was transmitted to mosquitoes and temporarily maintained in monkeys.

The patient lives in Essa Oke but came to Ife on 1st June to visit his son (Ijitona), mentioned above, who was ill with the same disease at the time. He has frequent attacks of fever and claims he had "yellow eyes" when a boy.

Present Illness.

This began on 7th June with a shaking chill, headache, and fever. When seen the following day the patient complained of severe frontal headache, photophobia, pain in the back, and insomnia. He is walking about, and shows no particular signs of prostration. His appetite is fair.

Physical Examination.

The eyes are markedly congested ; the sclerae are muddy and show indications of mild icterus. The tongue is typical of yellow fever, with black-brown coat and red edges and tip. The gums are negative. The heart is hypertrophied, and there is a mitral bruit. Arteriosclerosis and hypertension are fairly advanced. The lungs are normal. The liver and spleen are neither enlarged nor tender.

The blood is negative for parasites. The haemoglobin is 75 per cent. There is slight leucocytosis. The differential count is as follows : polymorphonuclears, 74 per cent. ; lymphocytes, 18 per cent. ; eosinophiles, 2 per cent. ; and mononuclears 6 per cent.

The albumin deposit is 1 gramme per litre of urine and equals one-tenth of the volume of urine boiled.

9th June.—The patient's temperature is 102·1° ; his pulse rate 72. He feels better but is lying down. There are slight evidences of prostration, but his general condition is fair. His headache is less severe, but pain in the spine persists. He has anorexia, nausea, and slight vomiting, but no photophobia or pain in the extremities. The eyes are markedly congested ; icterus is definite but not deep. The dorsum of the tongue is covered with a brown coat ; the edges and tip are red. The heart action is satisfactory ; the lungs are negative. The liver is enlarged but not tender. The spleen is not palpable. There is no abdominal tenderness.

The blood is negative for parasites. Leucocytes number 7,600. Albumin has increased to 2·5 grammes per litre of urine. Microscopic examination of a centrifugalized specimen of urine shows no casts.

10th June.—The patient's temperature and pulse are normal. He feels much better but is still somewhat prostrated and is lying down. He has a slight headache, but no other pains. Anorexia and nausea persist, but he is not vomiting. His eyes are moderately congested and icterus is still present. The tongue has a dark brown coat ; the edges and tip are red. The urine contains the same amount of albumin as on the previous day. A few granular casts are seen in a centrifugalized specimen.

11th June.—The patient's condition is very much improved. He has no headache and no pains, but he still has some anorexia, nausea, and vomiting ; and icterus continues. The Esbach test shows 0·5 gramme of albumin per litre of urine. No casts are seen in a centrifugalized specimen of urine.

12th June.—The patient is convalescent. He is not vomiting and his tongue is clearing. Albuminuria is unchanged. It is uncertain whether icterus is present.

The patient made an uninterrupted and satisfactory recovery. His urine was normal by 18th June.

6. *A very mild case which occurred in the same house and practically simultaneously with Case 5.*

Mrs. I. O. Ijitona, 24 years of age.

The patient was born in Ilesha and has lived in Ife 2½ years. She has three children, the youngest of whom was born 4 months ago when she was with her husband's family at Esso Oke. She returned to Ife 6 days before becoming ill. There is much *Aëdes* breeding in the neighbourhood in which she lives.

Present Illness.

This began 6th June with headache, moderate photophobia, anorexia, slight vomiting, and fever, but no chill and no pain in any part of the body.

Physical Examination.

On 6th June, the first day of illness, the patient's temperature is 103·6°, and her pulse rate 122 sitting. She is a strong, well developed woman, who does not look particularly ill and is up and about. Her eyes are slightly congested, but there is no icterus. Her tongue is typical of yellow fever—coated and pointed, with red edges

and tip. The gums are negative. The heart and lungs are normal. The liver is not palpable or tender. The spleen is slightly enlarged, but not tender. There is no epigastric tenderness.

The blood is negative for parasites. The haemoglobin is 70 per cent. There is slight leucocytosis. The differential count is polymorphonuclears, 82 per cent; leucocytes, 10 per cent.; mononuclears, 4 per cent.; and eosinophiles, 4 per cent. The urine contains no albumin and no casts.

7th June.—The patient feels practically well and looks better. Her temperature is 99·2°; her pulse rate 92. Headache and photophobia have disappeared. Anorexia persists, but vomiting has ceased. The eyes are slightly congested, but otherwise negative. The urine shows a faint trace of albumin, but no casts.

8th June.—The patient is better, looks well, is walking about, and is hungry. Her temperature, however, is 103, and her pulse rate 102. The urine shows a moderate cloud of albumin and an occasional cast.

Had it not been for the secondary elevation in temperature and the increase in albumin this case could only have been suspected of being yellow fever.

9th June.—The patient's temperature is 100·8°; her pulse rate 98. There is neither symptomatology nor icterus, but the tongue still has a heavy coat and red edges and tip. Urine examination by the Esbach technique shows a marked increase in albumin—1·5 grammes per litre. A small number of granular casts are seen in a centrifugalized specimen of urine.

The patient made an uneventful recovery. The urine showed the faintest trace of albumin on the 9th day and was entirely negative on the 13th day. There was no evidence of icterus at any time.

Percentage Tabulation of Symptoms.

In Table I are shown by days the percentages of cases in which various symptoms typical of yellow fever were present. The cases are divided into two classes, the mild and those of a more severe character. The former group is made up of thirty-three and the latter of seventy-one cases, but the line of demarcation between them is, of course, more or less arbitrary.

Fatal cases seen during the epidemics, approximately eighteen in number, are not included in the tabulation, but their symptoms and signs are discussed in the section of this report on clinical findings. Some of these patients were not in a condition to give authentic data when first seen; some were hidden away or removed to their native villages just before death; and the data on the whole are not as satisfactory as in the other classes.

During the study of the four epidemics all varieties of diseases were treated and large numbers of patients were seen in each of the towns. In addition to the cases definitely diagnosed as yellow fever and included in Table I (pp. 76 and 77), considerable numbers were observed with suggestive symptoms, but as there was some doubt as to their diagnosis they were excluded. This was also the case where the records, for various reasons, were insufficient to permit of reasonable analysis.

TABLE I. PERCENTAGE INCIDENCE OF THE MAIN SYMPTOMS AND SIGNS

Symptoms.	Per cent. of cases positive.	Mild Cases.									
		Percentages of cases positive by days.									
		1st.	2nd.	3rd.	4th.	5th.	6th.	7th.	8th.	9th.	10th.
Headache	100	100	82	72	41	28	12	12	6	3	3
Pain in back	84	83	83	67	42	26	16	9	6	3	0
Pain in extremities	76	52	57	47	31	15	6	6	3	0	0
Epigastric tenderness	50	15	8	34	36	23	18	10	10	8	0
Photophobia	75	80	61	38	11	4	4	0	0	0	0
Anorexia	84	75	71	74	52	31	18	6	3	0	0
Vomiting	50	28	20	23	13	13	13	3	0	0	0
Chill at onset	80										
Prostration	94	94	92	89	72	47	34	18	12	12	0
Eyes congested	90	77	77	55	50	38	28	24	12	0	0
Tongue suggestive*	88	87	90	86	80	44	30	20	15	0	0
Congested gums	29	4	4	11	10	7	10	7	3	3	0
Bleeding gums	6							3	3	3	
Icterus	54	0	4	36	48	40	31	29	20	20	10
Vomiting with blood	6	0	3	3	0	0	0	0	0	0	0
Enlarged liver	33	19	17	20	13	10	11	7	7	4	4
Tender liver	27	14	4	8	16	13	8	4	4	0	0
Temperature °F. (average)		102·1	100·1	99·8	99	98·7	98·7	98·3	98·3	98	97
Pulse rate (average)		106	85	81	77	75	71	71	68	68	56
Albumin (per cent. solidified)	100	·025	·05	·065	·07	·057	·078	·07	·022	·01	0
Casts	40										

* Typical tongue is small, coated, rather pointed, with red edges and tip.

OF YELLOW FEVER NOTED IN CASES AMONG WEST AFRICAN NATIVES.

Per cent. of cases positive.	Severe Cases (not fatal). Percentages of cases positive by days.										
	1st.	2nd.	3rd.	4th.	5th.	6th.	7th.	8th.	9th.	10th	
100	98	96	91	68	44	28	20	13	6	6	
90	84	78	78	66	54	37	31	16	6	5	
62	61	55	51	33	30	16	12	10	2	2	
81	48	54	64	68	71	56	52	43	20	20	11th 12th 13th 16 10 0
75	75	50	52	39	15	13	6	6	0	0	
93	90	90	90	80	72	62	44	22	22	14	11th 12th 13th 14th 9 9 6 0
76	35	32	56	51	43	22	5	0	0	0	
85											
100	90	94	94	90	87	80	71	54	42	33	11th 12th 13th 14th 15th 16th 17th 18th 26 15 16 10 9 9 7 0
90	90	83	87	85	74	70	38	17	12	0	
90	90	80	90	90	70	45	31	33	27	27	11th 18, 2 cases up to 16th day
54	5	13	25	23	30	30	30	24	21	6	11th 12th 3 2, 1 case 14th to 21st day
36	0	2	6	10	10	19	27	24	19	6	14th 15th 1 1
92†	0	4	38	72	92	80	80	80	63	60	11th 12th 13th 14th 15th 16th 17th 26th 60 40 40 35 35 30 14 0
10											2nd 3rd-4th 4th-5th 1 1 1
54	27	30	40	40	40	44	32	33	15	7	11th 15th 17th 3 1 1
50	17	23	28	29	33	29	18	19	10	0	11th 2
	102·6	102·1	101·0	100	99·5	99·2	98·9	98·9	98·4		3rd-4th 4th 6th 1 1 1
	105	93	83	76	74	68	66	65	65		4th 4th-5th 6th 1 1 1
100	·0157	·107	·265	·261	·286	·281	·265	·224	·161	·157	11th 12th 13th 14th 15th ·068 ·060 ·039 ·031 ·024
78											

† In only 66 per cent. of these was the icterus marked ; in remainder it was a mild tinting.

CLINICAL ANALYSIS.

Age.—The ages of the patients included in the study, as well as of those who died of yellow fever, are given in Table II.

<div align="center">TABLE II.</div>

<div align="center">AGE DISTRIBUTION OF PATIENTS OBSERVED.</div>

Town.	Age in Years.				
	0–9	10–19	20–29	30–39	40 and over.
Asamankese	5	14	25	4	2
Larteh	0	4	13	13	5
Suhum	0	7	15	3	0
Ife	0	2	6	3	1
Total	5	27	59	23	8

It will be noted that only five of the patients were under 10 years of age and that the total number below 20 years was only thirty-two. This indicates what is also true in epidemics among whites, that the great majority of missed cases was among young persons. That the disease was actually very prevalent among these persons is indicated by the results of protection tests with the sera from children of Asamankese, Larteh, and Ife. Of these sera, 40, 30 and 70 per cent. respectively showed protective properties.

An interesting finding in connection with the deaths from yellow fever is the fact that the great preponderance of these were in older persons. Among ninety-one patients under 30 years of age, only 8 or 9 per cent. died, while among thirty-one cases occurring in persons between 30 and 51 years, 10 or 32 per cent. terminated fatally. This difference is statistically highly significant $(P = 0.001)$.

Sex.—Of the 122 cases, eighty-five were in males and thirty-seven in females. The preponderance of males over females may be explained in part by the difference in habits of the two sexes and the greater liability of the former to exposure. The reluctance of females to consult a physician, especially a white physician, is, however, probably mainly responsible for the discrepancy.

Duration of Illness.—As the native is prone to eat his food, walk about the streets, and even at times to go to work in the acute stages of an illness, with high fever and heavy albuminuria, it was always rather difficult to determine the date of the onset of convalescence. In deciding this point we had to be guided by the results of physical examinations, urine analyses, etc., rather than by the statements or actions of those under observation. In general, the duration

in severe cases may be given as varying between 5 and 12 days—average 8 or 9 days—and in mild cases between 4 and 9 days—average 6 days. The duration in fatal cases varied between 4 and 11 days, while one case, mentioned above, did not terminate until the fifteenth day. The average day of death was between the seventh and eighth day, which is somewhat later than is usual in cases in Europeans seen in West Africa.

Temperature and Pulse Rate.—Under the conditions existing in the various towns in which the epidemics occurred, it was obviously impracticable to secure continuous temperature and pulse records of patients under observation or sufficiently comprehensive data to determine accurately the relationship between them. In the great majority of cases only a single reading was secured during the daily visit, while in many instances patients were not seen until the third or fourth day of illness, or sometimes later. Their most acute symptoms had accordingly subsided when they first came under observation, and the records fail to show the height of the fever. Therefore, though Table I gives the average temperature and pulse of all patients observed, the remarks below refer only to those seen early and followed throughout the course of the disease.

In mild cases, as a rule, fever reached a maximum on the first day of illness and subsided promptly thereafter. The average duration of pyrexia was only 3·3 days ; but in one case it persisted for 6 days. Among fifteen mild cases seen on the first day of illness, the maximum temperature was 104° (pulse rate 120) in one ; in three others it ranged between 103° and 104° ; while in the remainder it varied between 100·4° and 103°. In mild cases Faget's pulse was not in evidence, probably owing to early subsidence of the pyrexia ; but a relatively slow pulse was present in approximately 40 per cent. of these.

Among twenty-nine patients with moderately severe and severe cases, seen on the first and second day, four showed a temperature of 105° or more ; in three others the temperature was between 104° and 105° ; in eight it was between 103° and 104° ; while the remainder had milder degrees of pyrexia. In these cases fever was of longer duration than in those of the milder type, the average duration was 5·6 days, while in one case it persisted for 11 days. The typical saddle back curve, so frequently seen in cases of yellow fever among whites, was infrequent, the temperature having rather a tendency to be high on the initiation of illness and to fall gradually during the following days.

Much variation in the pulse rate was noticed in different patients, and this was probably due, in part at least, to nervous influences and fright. In some cases the rate was exceedingly rapid at the beginning of the illness and dropped promptly with the subsidence of fever. In approximately 67 per cent. the pulse rate was relatively slow in comparison with the degree of pyrexia, and Faget's sign was frequent among the severe and fatal cases.

Chills.—As shown in Table III, chills or chilly sensations were present at the beginning of illness in the great majority of mild, severe and fatal cases,

and they occasionally recurred during the course of the disease. As we had to rely entirely upon the statements of patients with regard to this symptom, it was difficult to differentiate between marked chilly sensations and a definite shaking chill.

TABLE III.

CHILLS.

Type of Case.	Definite Chill at Onset.		Chilly Sensations at Onset.		Negative.		No Record.
	No.	Per cent.	No.	Per cent.	No.	Per cent.	
Fatal	15	88	1	6	1	6	1
Severe	26	72	5	14	5	14	2
Moderately severe	16	53	8	27	6	20	3
Mild	16	50	9	28	7	22	1

Headache.—The symptoms most commonly seen in the West African native with yellow fever is headache. It was present in 100 per cent. of all cases studied and, with one exception, on the first day of illness. It was generally most severe at onset, gradually diminishing in intensity, and disappearing in a few cases by the second day and in the majority by the fifth or sixth day. However, in eleven cases it persisted for 8 days or more ; and in one instance the patient complained of this symptom for 17 days and well into convalescence. It was frequently limited to the frontal area, but in numerous instances it was generalized, involving the entire head and extending down into the spine.

This symptom is of great importance in the differential diagnosis of yellow fever ; for although it is present in many febrile diseases, it is rarely absent in this disease, and a negative history is presumptive evidence against the diagnosis. As a matter of fact, the diagnosis of yellow fever was tentatively excluded in a few cases observed recently in which this symptom was not present, and the negative diagnosis was later confirmed by protection test studies of the sera of the patients.

Bodily Pains.—Acute bodily pain was present in practically all cases studied, though two patients appeared to escape. In fatal and very severe cases it was, as a rule, generalized at the onset of illness but later became localized in the back, the limbs, and less commonly in the epigastrium. In the milder types of cases it was generally localized from the onset.

Pain in the back was, with the exception of headache, the symptom most

commonly noted in yellow fever in the West African native. It was complained of in 100, 90 and 84 per cent. respectively of fatal, severe, and mild cases. It was, as a rule, localized in the lower spine, but at times the dorsal region was involved, and occasionally the cervical region as well. It was usually most severe at onset and gradually tapered off during the first 5 days of illness; but in a few cases it persisted well into convalescence. From a diagnostic point of view it is probably more important than other subjective symptoms, for although it is not in evidence in all cases, its presence has great weight, as it is not particularly common in malaria or other febrile conditions seen in the West African native which might be confused with the disease under consideration.

Pain in the lower limbs, at times in the musculature but more often in the joints, particularly in the knees, was frequently complained of in all types of cases. It was often severe and at times excruciating to the extent of inhibiting voluntary movement on the part of the patient. The symptom was present in approximately 60 to 70 per cent. of all types of cases. Pain in the upper limbs was relatively rare.

Epigastric pain or tenderness was generally present in direct proportion to the severity of the illness, occurring in 87 per cent. of fatal cases, 80 per cent. of severe cases and 50 per cent. of mild cases. In fatal cases, the symptom generally appeared early and persisted up to the time of death. In mild cases, on the other hand, it appeared later during illness and was, as a rule, transient.

Photophobia.—It was somewhat difficult to be certain as to the occurrence of this symptom in cases that were not seen at the beginning of the illness, as the intelligence of the average native is not sufficient to permit him to differentiate between headache, pain in the head, and pain in the eyes. We generally judged as to the presence or absence of photophobia by the reaction of the patient when exposed to bright light, and it is believed, accordingly, that the figures given in Table I, *i.e.*, 75 per cent. for both severe and mild cases, are reasonably accurate. Our data on this point in fatal cases are insufficient to permit tabulation.

Anorexia.—Anorexia was a prominent symptom, being present in all fatal cases and in 90 and 75 per cent. respectively of those of severe and mild type. It generally appeared on the first day of illness and gradually decreased as fever subsided, but it frequently continued for a considerable length of time and well into convalescence.

Nausea and Vomiting.—Among eighteen cases with fatal termination on which the records are clear, nausea and vomiting were present in all except one. These symptoms appeared in some of the cases on the first day of illness, but in a number they were delayed until the third or fourth day or even later, when they persisted up to the time of death. Among thirty-seven patients, very severe cases, who recovered, these symptoms were present in thirty-four. They were frequently marked, but in some cases they were mild and lasted only

a few days.   Severe and continuous vomiting persisting throughout the course of the disease was present in only a relatively small number of instances.   Among thirty-two moderately severe cases, nausea and vomiting occurred in nineteen. The symptoms generally appeared at the onset of illness and continued only a few days, but in a small number they persisted into convalescence.   Though 50 per cent. of the mild cases exhibited these symptoms, vomiting was as a rule transient, occurring on only one day or sometimes on several days at some time during illness.

Melena and Haematemesis.—From what has already been said it will be obvious that the conditions existing in the towns in which the studies were made rendered it impossible to obtain accurate and conclusive data in connection with ejecta and stools.   As the natives defaecate in ruined buildings or in the bush or jungle, it was rarely possible to induce them to save stool specimens for observation, and it is quite probable that our records in connection with black vomit and melena, especially in severe and fatal cases, are inaccurate and that the presence of these symptoms may have frequently escaped detection.   Black stools and black vomit were actually observed in only a minimum number of cases : black stools were reported in two fatal and one severe case at Asamankese, and in one fatal and one severe case in Larteh ; and black vomit in four fatal and three severe cases in Asamankese, two fatal cases at Suhum, and one fatal case in Larteh.

Epistaxis.—Epistaxis was rarely seen, being present in only two cases each in Asamankese, Larteh and Su hum.

Congested and Bleeding Gums.—Congested and bleeding gums are frequently seen in native West African yellow fever patients, especially in severe and fatal cases.   However, these signs and other haemorrhagic manifestations would seem to be less frequent than in similar cases observed among white persons in West Africa and in the Americas.

Prostration.—This symptom was extreme in all fatal and severe cases and was present in varying degrees in almost all patients studied.   It was much more marked than in malaria or the usual febriculae commonly seen among natives, and was, together with a pulse of exceedingly low tension, a striking feature of the disease.

Congested Eyes.—This symptom was present in 90 per cent. of severe and mild cases and is important in the differential diagnosis of yellow fever in the West African native.   It was generally observed on the onset of illness, was frequently extreme, and persisted as a rule for 4 or 5 days.

Icterus.—Although jaundice, when present, is one of the most characteristic and important manifestations of yellow fever, it was absent in some severe cases and was noted in only 54 per cent. of the milder group of cases included in

the series. In the black man the symptom manifests itself primarily by a slight golden tinting of the sclerae ; when pronounced it is also visible in the conjunctivae, the mucous membranes of the mouth, and in rare instances in the lighter areas of the skin. It was present in varying degrees in all fatal cases observed and in 92 per cent. of cases of the severe type, as shown in Table I. However, among the latter, it was frequently exceedingly mild ; in only 66 per cent. was icterus deep. It appeared in a few mild cases and a few severe ones towards the close of the second day of illness, but more often made its appearance between the third and sixth day. In mild cases it was frequently transient, disappearing in 1 or 2 days, but in others it was present for a considerable period of time. In severe cases it was, as a rule, more prolonged and in many instances persisted for some time after recovery ; in one case it was still visible up to the twenty-sixth day. Errors in the differential diagnosis of yellow fever in the African are liable to be made because of the fact that few clinicians realize that the absence of jaundice does not by any means exclude this disease. On the other hand, some have been prone to make a diagnosis of yellow fever in cases with very deep icterus but without other cardinal symptoms, although, as shown in the appended table, such additional signs and symptoms are always present in severe cases of this disease.

Liver and Spleen.—Although the conditions under which examinations were made rendered it impossible to estimate accurately the size of the liver, slight to considerable enlargement of this organ, together with a varying degree of tenderness, was present in approximately 50 per cent. of severe and 30 per cent. of mild cases. This generally disappeared with the subsidence of the other symptoms.

A large hard spleen was frequently noted in patients in Asamankese and Suhum, but was rarely seen among those in Larteh. It was probably, as a rule, due to malaria and had no reference to the disease under consideration.

Tongue.—A tongue typical of yellow fever—small, more or less pointed, with a white or brown coat over the dorsum, and with definite red edges and tip—was present in many cases, and the appearance of this organ was at least suggestive in 90 per cent. of severe and 88 per cent. of mild cases. It was accordingly an aid in differential diagnosis in the cases observed and especially in excluding malaria, which is very prevalent among the natives of the Gold Coast.

Albuminuria.—This is probably the most striking feature of yellow fever in the African native ; and the urinary findings are the most important of all criteria in the differential diagnosis of the disease. Albuminuria was present in every case of the series, but varied markedly in intensity. The content of albumin in some cases amounted only to a light cloud, but in large numbers it was exceedingly heavy, and in a few practically the entire specimen became

solidified upon heating and the addition of acetic acid.   As shown in the
accompanying graphs, the average degree of albuminuria was in direct proportion

ALBUMINURIA, BY DAY OF DISEASE.

(Expressed in per cent. of total volume solidified by boiling.)

Mean value and the two extreme cases in each group.

to the severity of the disease, but there were many exceptions in individual
cases.   In numerous mild cases the albumin content was equal to one-tenth
of the volume of urine boiled, and in one it was double this amount.   On the
other hand, among some severe and moderately severe cases, where the average
was much higher, a maximum of only a slight precipitate was present in a few.

Albuminuria of a mild degree was sometimes present on the first day of
illness, but more often albumin made its appearance on the second or third day
and, increasing rapidly, reached a maximum on the fifth or sixth day.   From
this time on, in patients that recovered, there was a slow and gradual decrease
of albumin, and in the average of mild and severe cases the specimens were

clear by the ninth and sixteenth day respectively. In a limited number of mild cases albuminuria was exceedingly transient, lasting only for 1 day or 2 days.

Microscopical Examination of Urine.—Centrifugalized specimens of urine were used exclusively in making microscopic examinations, which were carried out daily as far as possible. Casts, more often of a fine or coarsely granular type, were present in approximately 40 per cent. of mild cases ; but they were not as a rule numerous, and they had a tendency to be transient, being found on some days and frequently disappearing even when heavy albuminuria persisted. In moderately severe and severe cases they were much more frequently seen (in 78 per cent.) in greater number and persisted for a considerable period of time. They generally made their appearance on the third or fourth day of illness. In jaundiced cases the casts were often deeply bile-stained.

Red blood cells were, as a rule, present in small numbers in 25 per cent. of mild and severe cases. White blood cells were also frequently seen, and pus was present in a few cases ; but these findings have, of course, no reference to the disease under consideration.

Blood Examinations.—As far as practicable, blood examinations were made in every case ; smears were searched for malaria and other parasites, and differential counts were carried out. In a certain number, white and red blood-cell counts and a haemoglobin determination were also made. These blood examinations made it possible to clear up the differential diagnosis in some cases which might have been considered as mild yellow fever if judged from clinical data alone, and definitely to eliminate relapsing fever in all cases included in the series. In this connection, it is interesting to note that while the epidemic in Asamankese was still in progress word was received of the presence of a fatal illness among the natives of Akwatia, 21 miles north. Superficial examination of some of the patients rather suggested yellow fever, but this disease was promptly excluded in all through blood examinations, which showed large numbers of *S. recurrentis*, and by postmortem examination in fatal *spirochaeta* cases, which revealed pathological changes dissimilar from those seen in the yellow fever cases in Asamankese. Toward the close of the epidemic in the latter town, one case of relapsing fever was actually diagnosed.

Time did not permit of making more than a single blood examination in the majority of cases, and it is therefore impossible to generalize on the blood picture in yellow fever among Africans from day to day. The records, however, suggest that there was in some cases a mild leucocytosis at onset followed by a moderate to marked leucopenia. In other cases the count was within normal limits throughout ; and in considerable numbers moderate to marked leucopenia was present. Among ninety-three patients whose blood was examined on one or two occasions, and generally early during illness, that of

solidified upon heating and the addition of acetic acid.   As shown in the accompanying graphs, the average degree of albuminuria was in direct proportion

ALBUMINURIA, BY DAY OF DISEASE.

(Expressed in per cent. of total volume solidified by boiling.)

Mean value and the two extreme cases in each group.

to the severity of the disease, but there were many exceptions in individual cases.   In numerous mild cases the albumin content was equal to one-tenth of the volume of urine boiled, and in one it was double this amount.   On the other hand, among some severe and moderately severe cases, where the average was much higher, a maximum of only a slight precipitate was present in a few.

Albuminuria of a mild degree was sometimes present on the first day of illness, but more often albumin made its appearance on the second or third day and, increasing rapidly, reached a maximum on the fifth or sixth day.   From this time on, in patients that recovered, there was a slow and gradual decrease of albumin, and in the average of mild and severe cases the specimens were

STUDIES IN MICROCATAPHORESIS.

II.—THE ELECTRIC CHARGE OF HAEMOFLAGELLATES.

BY

J. C. BROOM,

H. C. BROWN

AND

C. A. HOARE.*

(*From the Wellcome Bureau of Scientific Research, London.*)

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The greater part of the literature on the determination of the electric charge of microorganisms is devoted to bacteria and only a very few observations deal with spirochaetes and haemoflagellates of the genera *Trypanosoma* and *Leishmania*. Most observers seem to be agreed that bacteria are negatively charged, but there is a considerable amount of controversy as regards the electric charge of spirochaetes and haemoflagellates. In the case of spirochaetes, KROSS and ZUELZER (1932) have recently undertaken an exhaustive critical revision and have demonstrated conclusively that under normal conditions spirochaetes (genera *Leptospira* and *Treponema*) bear a negative charge and do not differ in this respect from the bacteria.

The position as regards trypanosomes and leishmanias however so far remains unsettled. The total number of papers bearing on the electric charge of these protozoa does not appear to exceed half a dozen. COMMANDON (1911)† was apparently the first to observe the electrophoresis of trypanosomes (species not named) : these according to him migrate to the cathode, while the red corpuscles move to the anode. COMMANDON found that *Treponema recurrentis* and *T. gallinarum* behaved like the trypanosomes, whereas *T. pallidum* migrated to the anode.

TRAUBE (1912, 1912a, 1915) also stated that trypanosomes (unspecified) migrate to the cathode. HÖBER (1914) however, was unable to confirm TRAUBE's results : HÖBER examined the cataphoresis of the blood forms of *Trypanosoma brucei* and *T. equiperdum* and found that both migrated to the anode.

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*We wish to thank Mr. R. J. REED and Mr. J. A. HAYNES for their valuable help throughout these experiments.

†In an unpublished communication quoted by TRAUBE (1912, 1915).

threc showed leucocytosis and that of twenty-nine showed leucopenia ; the remainder were within normal limits.

Polymorphonuclear cells were relatively increased in a few cases and greatly reduced in many. Among seventy-seven cases studied, the percentage of polymorphonuclears was between 20 and 30 in two, between 30 and 40 in five, between 40 and 50 in sixteen, between 50 and 60 in fifteen, between 60 and 70 in nineteen, between 70 and 80 in sixteen, and over 80 in four.

Lymphocytes were correspondingly increased in many cases.

SUMMARY.

Four epidemics of yellow fever observed among natives in cities of the Gold Coast and Nigeria, West Africa, are described ; and the main clinical manifestations of the disease in mild, severe, and fatal cases are presented.

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STUDIES IN MICROCATAPHORESIS.

II.—THE ELECTRIC CHARGE OF HAEMOFLAGELLATES.

BY

J. C. BROOM,

H. C. BROWN

AND

C. A. HOARE.*

(*From the Wellcome Bureau of Scientific Research, London.*)

The greater part of the literature on the determination of the electric charge of microorganisms is devoted to bacteria and only a very few observations deal with spirochaetes and haemoflagellates of the genera *Trypanosoma* and *Leishmania*. Most observers seem to be agreed that bacteria are negatively charged, but there is a considerable amount of controversy as regards the electric charge of spirochaetes and haemoflagellates. In the case of spirochaetes, KROSS and ZUELZER (1932) have recently undertaken an exhaustive critical revision and have demonstrated conclusively that under normal conditions spirochaetes (genera *Leptospira* and *Treponema*) bear a negative charge and do not differ in this respect from the bacteria.

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We have fitted up an apparatus similar to that used by SZENT-GYÖRGYI (1920) with non-polarizable electrodes and although on some occasions we have obtained the correct reading for the sign of the charge of the trypanosome, on others the sign of the charge appeared to be reversed. Our findings in this respect are in accordance with those of KROSS and ZUELZER (1932) who carried out a series of parallel tests in closed and open cataphoresis cells and came to the conclusion that electrophoresis in an open cell is not even of any qualitative value. They found that normal *Bact. coli* and spirochaetes observed in an open cell appeared at times to be positively and at other times to be negatively charged. Subsequently in a closed cell all were shown to be negatively charged.

In view of these discrepancies and because of the importance of the subject in helping to elucidate certain problems connected with the chemotherapy and serology of trypanosomiasis and leishmaniasis we decided to make a critical revision of the cataphoretic behaviour of the haemoflagellates responsible for these conditions. With this end in view we have examined all the strains of these protozoa available.

TECHNIQUE.

We have described elsewhere in detail the technique which we use (BROWN and BROOM, 1936) and have drawn attention to certain essential points. We have shown that the results are valueless unless these precautions are adopted.

Previous observers have suspended the trypanosomes under observation in a number of different media and we intended to employ each of these media in the present study. We found, however, that the current passed by these media in our cataphoresis cell was sufficient to cause polarization of the electrodes. To overcome this, voltage reduction was not satisfactory because the migration rate became too slow for a reading to be made.

We finally employed a medium of pH 7·2 consisting of one volume of physiological saline and three volumes of 4 per cent. glucose in freshly boiled distilled water and in this paper the sign of the charge of flagellates refers to their behaviour under these conditions.

The solution was prepared fresh daily.

Throughout these experiments a cataphoresis cell of width 5 mm. and depth 0·75 mm. was used : this enabled a voltage drop of 9 volts per centimetre to be employed.

As we have shown in our previous paper (BROWN and BROOM, 1936) it is essential that observations in such a narrow cell be made in the Komagata stationary layer. The equation from which this position is arrived at differs from that more generally used by taking the width/depth ratio (K) of the cell into consideration. The necessity for this has been shown by KOMAGATA (1933).

The ratio (K) is obtained as follows. The width (W) is measured in millimetres by means of a caliper micrometer gauge before the cell is completely

assembled. As the depth (D) for the ratio (K) must also be expressed in millimetres, the combined thickness of the upper and lower plates, measured with a micrometer when the plates are first cut, is subtracted from the total thickness of the completed cell.

Since the position of Komagata's layer is calculated in terms of the graduations of the fine adjustment, the depth (2b) of the cell must be determined in this way also. When these measurements have been obtained the position of the stationary layer can be calculated with the help of Table 1, which shows

TABLE I.

SHOWING STATIONARY LEVEL AS FRACTION OF TOTAL DEPTH IN CELLS OF DIFFERENT K VALUES.

K Value.	6	7	8	9	10	12	15	20	25	50	100
Level	0·182	0·186	0·189	0·192	0·194	0·197	0·199	0·202	0·204	0·208	0·210

the factor by which the depth (2b) must be multiplied to give the position of the Komagata stationary level as a certain number of graduations above the upper surface of the lower plate of the cell.

For example, a certain cell had a depth (D) of 0·81 mm., and a width (W) 4·85 mm., giving the width/depth ratio (K) = 6·0. The depth (2b) measured by the graduations on the fine adjustment was 598 divisions.

From the table the factor for a (K) ratio of 6·0 is 0·182, hence the lower stationary layer is 598 × 0·182 = 109 divisions above the upper surface of the lower plate of the cell.

THE DETERMINATION OF CHARGE.

The cataphoresis cell is placed in position on the microscope as described in our previous paper (BROWN and BROOM, 1936).

A small quantity of blood from the tail of an infected mouse or rat is added to 5 c.c. of the freshly prepared glucose-saline solution mentioned above. The amount of blood added depends on the heaviness of the infection but should not exceed 20 c.mm. In the case of cultures of trypanosomes and flagellates the water of condensation is used instead of blood.

The cell is filled with this suspension, the current switched on and the direction of movement of the trypanosomes in the stationary layer is noted.

A voltage drop of approximately 9 volts per centimetre will be found convenient. Readings are taken with the current passing in both directions by means of a reversing switch.

No attempt has been made to estimate the actual migration rates of the flagellates, but only the sign of the charge has been considered.

The following haemoflagellates were used for these observations.*

Trypanosoma lewisi (blood and culture forms).

T. cruzi (blood and culture forms).

T. melophagium (sheep trypanosome : insect forms).

T. evansi
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Leishmania donovani (culture forms).

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L. agamae (cultures from the blood of lizard *Agama stellio*, Palestine).

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The Charge of the Insect Forms.

We have examined the charge of the developmental stages of the sheep-trypanosome, *Trypanosoma melophagium*, in its insect-vector, the ked, *Melophagus ovinus*. The flagellates, which were obtained from a pool of the intestinal contents of fifteen keds, proved to be negatively charged.

The Charge of the Cultural Forms.

We have repeatedly examined the charge of cultural forms of *Trypanosoma lewisi* and *T. cruzi* and have consistently found it negative.

As regards other trypanosomes we have so far obtained growth in culture only in the case of a strain of *T. rhodesiense* which was originally negatively charged in the mouse. These cultural forms remained negatively charged through a period of 3 months.

All the flagellates of the genus *Leishmania* named above have also been negatively charged in culture.

The Charge of the Blood Forms.

At the beginning of this investigation a considerable number of observations were made on the charge of various species of trypanosomes in mice, rats and guineapigs. It was found that some strains were positive, some negative, and that others showed both positive and negative individuals in the same animal.

It was not until we examined the same animals day after day that we began to understand how these irregular findings arose. In the case of mice our usual procedure was to inoculate six with a trypanosome of known charge and examine the blood of these animals daily. Further batches of six mice were sub-inoculated at varying stages of the infection for a number of passages and these also were examined daily.

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T. cruzi.—Owing to the difficulty of producing sufficiently heavy infections in laboratory animals, only one strain of this trypanosome has been studied. This has proved to be negatively charged.

T. evansi.—A laboratory strain maintained since 1922. Over a considerable number of passages in mice, daily observations of this strain showed the charge to be positive. As a rule the infection proved fatal in 8 or 9 days, but in one instance the strain reappeared in a mouse after an apparently spontaneous cure. The charge of the relapse trypanosomes was negative. From this negative variant more than twenty passages in mice have been made up to date without any alteration in the sign of the charge. These changes are diagrammatically represented in Fig. 1.

T. congolense.—A laboratory strain maintained since 1933. Because of the irregularities in the day-to-day infection which this organism produces in mice it proved difficult to take daily observations. We have not considered these changes in the number of circulating trypanosomes as relapses because the flagellates never disappeared completely from the peripheral blood. Intermittent observations when the infection was sufficiently intense showed that there was no constancy in the sign of the charge.

T. brucei.—A laboratory strain (UD) maintained for the last 5 years.

FIG. 1.
T. evansi.

SHOWING THE REVERSAL OF THE SIGN OF THE ELECTRIC CHARGE OF TRYPANOSOMES WHEN THE INFECTION RELAPSED IN A MOUSE.

This is the normal strain from which the tryparsamide-fast strain, to be described later, was obtained.

Through the first four generations in mice it invariably remained positively charged. During the fifth generation a change took place from positive to negative, 3 days after it had been found to be positive. Passages from the positive and negative variants of this generation remained positive and negative respectively.

T. gambiense.—Laboratory strain (M) maintained since 1932. As a rule infection with this strain proved fatal to mice in 7 to 10 days.

The first batch of mice inoculated with this strain all showed positively charged trypanosomes. Eight serial passages subinoculated in the early stages of infection bred true, *i.e.*, they remained positively charged.

One mouse of the original batch showed apparently spontaneous cure on the 8th day of infection and then relapsed on the 13th day. The infection was not heavy enough for the determination of the charge until the 20th day

when the trypanosomes were found to be negatively charged. A mouse sub-inoculated on the 20th day also showed negatively charged trypanosomes. Mice had also been inoculated on the 13th day. These likewise showed negative trypanosomes which remained negative through seven further passages.

The trypanosomes in one mouse of this batch subinoculated on the 13th day showed a second reversal of charge back to positive after a spontaneous cure. This positive variant remained positive in further serial passages. These changes in charge can be followed in Fig. 2.

FIG. 2.

T. gambiense.

SHOWING HOW TRYPANOSOMES REVERSE THE SIGN OF THE ELECTRIC CHARGE WHEN THE INFECTION RELAPSES IN MICE.

It will thus be seen that with the polymorphic trypanosomes also reversal of charge takes place when relapse occurs after spontaneous cure in mice.

T. rhodesiense.—This is the normal strain from which the tryparsamide-fast and atoxyl-fast strains, to be described later, have been derived. It is a strain maintained in laboratory animals for 12 years.

This strain has so far proved consistently negatively charged in mice. It is so virulent that only a single observation is usually possible during each passage and no relapse has occurred.

Drug-fast Strains.

Professor WARRINGTON YORKE very kindly supplied us with three drug-fast strains of trypanosomes. (1) *T. rhodesiense*, tryparsamide-fast ; (2) *T. rhodesiense*, atoxyl-fast ; (3) *T. brucei*, tryparsamide-fast.

Both the tryparsamide-fast and atoxyl-fast strains of *T. rhodesiense* are negatively charged. Thus they do not differ from the normal strain from which they were derived.

The tryparsamide-fast strain of *T. brucei* was negatively charged when first examined and thus differed from the normal strain. This cannot be looked upon as important because a negative variant developed from the normal (non-drug-fast) strain of this organism as already described. Further, the tryparsamide-fast strain showed both positive and negative variants. On passage in mice the results with this particular trypanosome do not conform to our usual finding in mice which is that reversal of the sign of the charge is associated with spontaneous cure and subsequent relapse. In this single instance reversal apparently occurred during the course of infection.

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The Effect of the Host on the Sign of the Charge.

The nature of the host is apparently of importance in the satisfactory performance of the adhesion test for the serological differentiation of trypanosomes (DAVIS and BROWN, 1927). We, therefore, decided to test the effect on the charge of inoculating infected mouse blood into guineapigs and rats.

A guineapig was inoculated with the *negative* variant of *T. evansi* from a mouse. Six weeks later trypanosomes appeared in the peripheral blood and these, inoculated into mice, were *positively* charged. The guineapig showed spontaneous cure but relapsed 2 weeks later. When examined on this occasion the trypanosomes were found to be *negative*. These changes are shown in Fig. 3. On another occasion two guineapigs were inoculated with the same negative variant and two others with the positive original strain. Two months later the pigs inoculated with the *negative* variant showed *positively* charged trypanosomes, whereas those inoculated with the positive strain showed trypanosomes of which some were *positive* and some *negative*.

In another experiment rats were inoculated with the normal strain of *T. rhodesiense* which was *negative*. When the infection appeared the trypanosomes were *positively* charged.

On the other hand rats, infected with the tryparsamide-fast and atoxyl-fast strains of the same organism, showed trypanosomes in each case which had remained *negatively* charged.

FIG. 3.

T. evansi.

SHOWING THE REVERSAL OF THE SIGN OF THE ELECTRIC CHARGE OF TRYPANOSOMES BY CHANGE OF HOST AND SECOND REVERSAL WHEN THE INFECTION RELAPSED

Simple Method for Determination of Charge.

All the determinations of electric charge referred to above have been made in our microcataphoresis cell.

As we have previously pointed out the calculation of the position of the stationary layer is valid only when the mechanics of the fine adjustment of the microscope are satisfactory.

From our experience of a large number of microscopes of British and foreign manufacture the degree of movement of the objective practically never corresponds to the number of divisions on the fine adjustment. This means that in the majority of instances the calculated number of revolutions of the fine adjustment will not focus the microscope in the true stationary layer. Therefore, unless a specially constructed microscope is obtainable, the determination of charge by microcataphoresis is impossible.

To overcome this difficulty we have evolved a simple technique for finding the charge of trypanosomes.

Our test consists in adding 0·002 c.c. of infected blood to 0·3 c.c. of 10 per cent. normal saline in 4 per cent. glucose, prepared by adding one volume of normal saline to nine volumes of 4 per cent. glucose. Interaction is allowed to occur for half-an-hour at room temperature and the suspension is then observed under the microscope with a one-sixth inch objective.

When the trypanosomes are positively charged they will be seen firmly adherent to the red cells either singly or in groups, whereas negatively charged trypanosomes are perfectly free and non-adherent. When both types are present in one infection we have found that instead of 100 per cent. adherent or free a varying percentage show adhesion. This is shown semi-diagrammatically in Fig. 4.
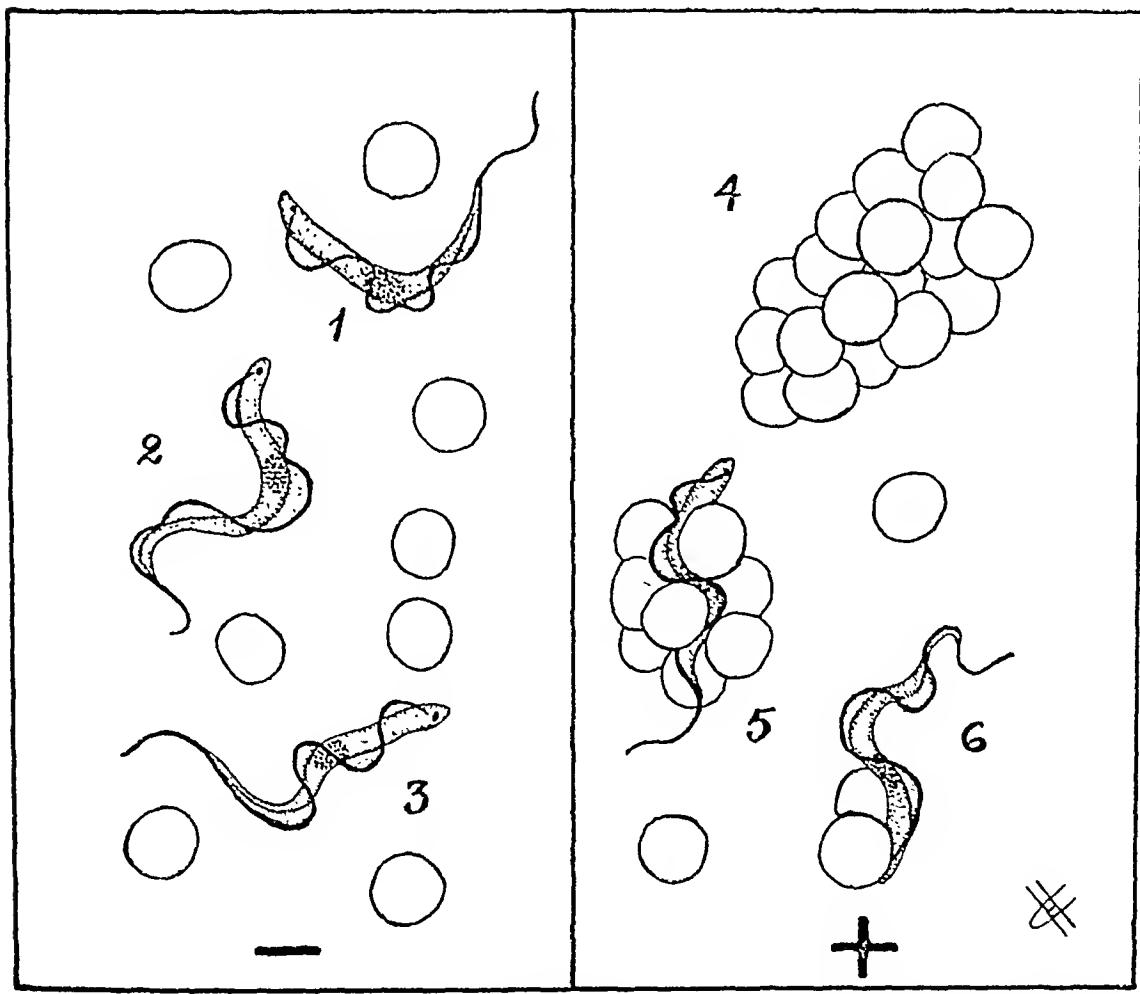
FIG. 4.—The Behaviour of Negative and Positive Variants of *Trypanosoma gambiense* in Glucose-Saline.

1, 2, 3. *Negative variant* : trypanosomes and red blood-corpuscles free.
4, 5, 6. *Positive variant* : various degrees of attachment of red cells to the trypanosomes (in 4 the trypanosome is completely overladen with red cells).
(Semi-diagrammatic figures drawn from stained preparations with the aid of a camera lucida).

We decided that a single concentration of 10 per cent. saline in 4 per cent. glucose solution gives invariably a satisfactory differentiation between positively and negatively charged trypanosomes after carrying out a series of tests in which varying salt concentrations were used.   The result of one complete test is shown in Table 2.   In this actual case the 10 per cent. solution does not show that

TABLE II.

PERCENTAGE OF TRYPANOSOMES ADHERENT TO RED CELLS IN VARYING SALT CONCENTRATIONS.

Charge of Trypanosomes under Observation.	Concentration of Saline.				
	0	10 per cent.	20 per cent.	30 per cent.	40 per cent.
Positive	100	98	100	22	0
Negative	0	1	0	0	0
Mixed	46	58	46	20	0

this is the optimum concentration but this generally gives the best correlation with the results in the cataphoresis cell.   Fifty trypanosomes were observed in each case and recorded as the percentage adherent, *i.e.*, positively charged.

The above results are of interest taken in conjunction with the contention of SZENT-GYÖRGYI that positively charged trypanosomes should adhere to the negatively charged red cells under normal conditions.   Since this does not occur, he concluded that the serum proteins had a " protective " action.   To overcome this possible protection he suspended infected blood in isotonic glucose solution to precipitate the globulins.   He then found that adhesion took place.

If this is the true mechanism, removal of the proteins by washing should allow adhesion to take place when red cells and trypanosomes are re-suspended in saline.   We have found however that adhesion does not occur under these conditions even after repeated washing in the centrifuge.

PHYSIOLOGICAL DIFFERENCES BETWEEN POSITIVELY AND NEGATIVELY
CHARGED TRYPANOSOMES OF THE SAME SPECIES.

(1) *Susceptibility to the Action of Arsenicals.*

We have already described the reappearance of trypanosomes of altered charge in mice infected with *T. evansi* after apparently spontaneous cure.   It is with these positively and negatively charged variants, originally derived from the same mouse, that we have tested the susceptibility to arsenicals.   It must

be realized that the resistance of these different trypanosomes to drugs is purely relative and that a suitable dose must be chosen to demonstrate it. Too small a dose has no effect on either variant and a sufficiently large dose will naturally sterilize in both cases.

The behaviour of the positive and negative variants when treated with tryparsamide is shown in the following experiments. The procedure is that adopted by PEARCE (1919) in the original test of tryparsamide. The number of trypanosomes in two mice was estimated by haemocytometer counts so that mice might be infected with the same number of organisms. Suitable dilutions of the heart blood were made in glucose-saline to contain 180,000 trypanosomes in 0·1 c.c. Fifty-five mice were inoculated with this number of the positive variant and the same number with the negative variant. All these mice weighed between 19 and 21 grammes.

Twenty-four hours later fifty mice of each batch received an intraperitoneal inoculation of 20 mg. of tryparsamide in 1 c.c. of distilled water. The remaining ten mice were given 1 c.c. of saline.

The mice were examined daily. At the end of the 1st week thirty-eight of the fifty mice injected with the positive variant were still free from infection whereas only seventeen negative variant mice remained uninfected. At the end of the 2nd week, the numbers were thirty and five respectively. The probability of these results being due to chance is about 10,000 : 1 and 1,000,000 : 1 against, respectively.

In another experiment two batches of twenty mice were similarly treated, the dose of tryparsamide in this case being 30 mg. In the mice receiving the negative variant the numbers uninfected after 1, 2, 3 and 4 weeks were twenty, thirteen, eleven and eight respectively. Not a single mouse showed infection with the positive variant throughout the whole course of the experiment. The probability of these results being due to chance rises steadily to 5,000 : 1 against.

It is obvious from these experiments that the positively charged variant is distinctly more susceptible to the action of the negatively charged ion of tryparsamide.

So far we have not had the opportunity of testing the converse of this experiment using a positively charged effective ion.

(2) Serological Differences.

The experiments which we have performed up to date on the serology of these variants can only be considered as preliminary. We have used both the adhesion test of DAVIS and BROWN (1927) and the protection test described by SCHILLING and NEUMANN (1932).

In the case of *T. evansi*, a serum prepared against the positive variant was found to cause 100 per cent. adhesion with the homologous trypanosome

but gave no adhesion with the negatively charged relapse variant. On the other hand a serum prepared against the negative variant caused 100 per cent. adhesion with both variants.

Protection tests were carried out with these two sera with the result that the " positive " serum protected against a positively charged trypanosome but not against the negatively charged one. The " negative " serum protected against both variants. The protection and adhesion tests are, therefore, in accordance in these experiments.

The results of the adhesion test with *T. gambiense* are shown in Table 3.

TABLE III.

ADHESION TEST *T. gambiense.*

Trypanosome and Charge.	Antiserum.			Normal Serum.
	Anti-primary.	Anti-relapse A.	Anti-relapse B.	
Primary (+)	100	0	99	0
Relapse A (—)	2	99	0	0
Relapse B (+)	99	0	100	0

The numbers indicate the percentage of trypanosomes showing adhesion.

Three strains were used, the original or primary, the first relapse A, the second relapse B (cf. p. 92). Here it will be seen that the primary strain and the B variant are serologically identical whereas the A variant is serologically distinct from the other two.

In protection tests with these three sera each serum protected only against its homologous trypanosome which is not in accordance with the adhesion test.

It is obvious that considerably more work must be done on this subject before a definite conclusion can be reached.

DISCUSSION.

Our experiments have shown that the sign of the electric charge of trypanosomes in laboratory animals is not constant but may be either positive or negative. We are of opinion that these differences in the sign of the charge are due to relapses having taken place in the previous history of the strain.

These findings appear to us to have an important bearing on the chemotherapy of the disease as we have shown that a positively charged trypanosome *in vivo* is considerably more susceptible to the action of tryparsamide than a negatively charged variant of the same species.

If our contentions are found to hold with regard to the alterations in charge in trypanosomiases of man and domestic animals and in leishmaniases, the selection of a suitable drug would seem to be a matter of great importance in treatment of these diseases. Similarly, in estimating the chemotherapeutic index of drugs on trypanosomes the sign of the charge of the trypanosome will obviously affect the results. The sign of the charge can easily be determined by means of the salt concentration test which we have devised.

We have only touched upon the serological problems involved when dealing with positively and negatively charged variants. Further work on this subject is in progress; we consider it probable that many of the difficulties which have been encountered when using serological tests are due to these strains of different electric charge.

If further experimental work confirms our observation on *T. melophagium* that the developmental stages in the invertebrate host are negatively charged it would lend additional support to the view that the evolution of trypanosomes in culture is an imitation of their natural development in the invertebrate host (cf. HOARE, 1923), since the cultural forms of all the haemoflagellates studied have also been negatively charged.

SUMMARY.

1. The electric charge in glucose-saline, pH 7·2, of the insect, cultural, and blood forms of a number of trypanosomes and other haemoflagellates have been examined.

2. The insect and cultural forms have been found to be negatively charged.

3. The blood forms may be positively or negatively charged. In some instances, both positively and negatively charged trypanosomes may be present in the blood of an infected animal simultaneously.

4. No alteration in the sign of the charge takes place in continuous progressive infections during serial passage in mice for more than thirty generations.

5. However, the sign of the charge of the trypanosomes which reappear after apparent spontaneous cure and subsequent relapse in mice is invariably the opposite of that carried by the original strain.

6. Alteration of the host can also change the sign of the charge of trypanosomes.

7. A simple "salt-concentration test" for determining the sign of the electric charge of trypanosomes is described.

8. In the case of *T. evansi* it is shown that the "positive" variant is

distinctly more susceptible to the action of tryparsamide than the " negative " one.

9. " Positive " and " negative " variants also differ serologically.

10. The importance of the electric charge in the chemotherapy and serology of trypanosomes is briefly discussed.

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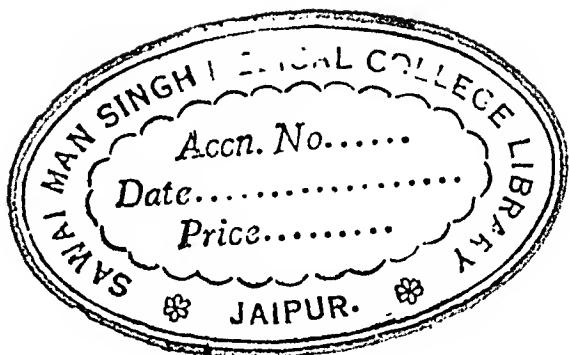
THE MODE OF ONSET OF THE MALARIA EPIDEMIC IN CEYLON.

BY

COLONEL C. A. GILL, M.R.C.P. (LONDON), D.P.H., D.T.M. & H., I.M.S. (RETD.).

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In a paper read at a meeting of the Royal Society of Medicine (Section of Tropical Diseases) on March 5th, 1936, Lt.-Col. S. P. JAMES (1936) stated that he and his co-workers at Horton had arrived at the conclusion, as the result of the scrutiny of some temperature charts and records of blood examinations recently received from Ceylon, that the malaria epidemic in that island started gradually and was associated with a slow, steady increase of primary infections.

On the other hand, in a paper published in the February number of the *Transactions of the Royal Society of Tropical Medicine* (GILL, 1936), I advanced the hypothesis, on the basis of a prolonged study of the epidemic in Ceylon, that the epidemic started suddenly by means of an " epidemic of relapses."

This hypothesis, which, if correct, is pregnant with far-reaching implications, is admittedly at present of a tentative nature, but it was not put forward hastily and without substantial evidence in support of its accuracy.

Colonel JAMES made no reference to this hypothesis or to the reasons that led to its adoption, but in view of his diametrically opposed conclusions it becomes necessary to examine the evidence upon which Colonel JAMES bases his opinion.

(1) MODE OF ONSET: SUDDEN OR SLOW.

Colonel JAMES states that the epidemic started gradually, but he qualifies this conclusion by remarking that it became explosive (for some unexplained reason) about the 6th week.

The evidence upon which this conclusion is based is partly clinical and partly parasitological but, before considering it, it may be well to recall that the sudden onset of regional epidemics of malaria has always been regarded as one of their most characteristic features. It was on this account that S. R. CHRISTOPHERS (1911), as the result of his study of the malaria epidemic in the Punjab in the year 1908, proposed the name of " fulminant malaria " for this type of epidemic, the word " fulminant " being defined in the *Oxford Dictionary* as something " developing suddenly."

The epidemic studied by me in the Punjab (GILL, 1928) exhibited fulminant features, and, so far as is known, the extremely abrupt onset of these epidemics has never hitherto been questioned.

On this account it was not considered necessary to describe in great detail the similar mode of onset of the Ceylon epidemic, and it was thought that the facts, figures, and charts given in my paper (GILL, 1936) sufficed to establish the extremely sudden onset of the epidemic.

To supplement this evidence I now reproduce in Table I the relevant part of a table contained in the official report on the Ceylon epidemic by Dr. R. BRIERCLIFFE (1935), Director of Medical and Sanitary Services, Ceylon, which shows the weekly number of attendances at twenty·hospitals and dispensaries in the severely afflicted district of Kurunegala during the months August to December, 1934, inclusive.

It will be seen that at almost all these hospitals and dispensaries the weekly number of attendances, after being remarkably constant for several months before the epidemic, underwent a large rise (often doubled or more) during the week ending November 3rd ; this is marked in the official report with an asterisk, which a footnote explains indicates " the start of the epidemic."

The figures given in Table I thus show that over an extensive area in this large district the epidemic started, so far as sickness is concerned, in the same week, but it was only as the result of examining personally the daily figures of attendances of a large number of hospitals and dispensaries in the epidemic area that the new and striking fact was brought to light that the time of onset of the epidemic could often be fixed to a day, this day frequently being the same day or a nearly related day throughout a wide area.

It should be pointed out that the weekly figures in respect of attendances do not fully reveal this fact. Thus, if the daily figure, after being constant say at 100 (= 700 weekly) for several months, is suddenly trebled on the *first* day of a week, the total figure for that week would rise to 2,100. But if in that week the daily attendance did not increase until the *fifth* day, the total figure for that week would rise from 700 to 1,300 only, and would not reach 2,100 till the next week. In the first case, therefore, the weekly figures for three consecutive weeks would read, 700, 2,100, 2,100 ; and in the second case 700, 1,300, 2,100. From a study of these weekly returns it would, probably be admitted that, in the first case the epidemic commenced

TABLE I.

KURUNEGALA DISTRICT.

Weekly Attendances at each Hospital and Dispensary showing rise and development of the Epidemic.

1931.

	Aug.			Sept.					Oct.				Nov.				Dec.				
	11	18	25	1	8	15	22	29	6	13	20	27	3	10	17	24	1	8	15	22	29
Hospitals.																					
Dambadeniya	701	680	711	655	616	498	546	700	759	637	810	892	1863	2460	2470	2152	3690	3562	7831	10392	6175
Dandegamuwa	1158	1385	1246	1273	1106	1199	1295	1071	1016	1097	1139	1146	1715	1956	2311	2319	3097	5259	9794	10123	7931
Kurunegala	1136	1074	1081	1018	1046	1028	1020	986	1072	961	1113	1385	2763	2909	2557	2648	2911	3022	3275	2959	2116
Nikaweratiya	367	410	341	345	321	319	316	426	378	373	369	353	496	922	1382	1200	1269	1162	1577	2023	1759
Ridigama	482	540	522	470	446	535	607	656	490	699	697	550	1011	1838	1881	1419	1335	1309	1385	1940	1012
Dispensaries.																					
Batalagoda	167	195	160	158	173	193	213	213	192	181	232	281	668	1270	1158	1311	1156	1504	1107	1561	889
Bingiriya	508	590	472	520	508	437	509	555	521	601	621	607	811	1233	1518	1037	1231	1437	2615	4367	3740
Dodangaslanda	96	81	50	108	136	163	134	139	159	225	222	200	513	700	721	556	551	885	818	990	755
Galgamuwa	305	207	320	265	318	251	301	331	411	396	372	330	494	875	1287	1294	1089	1029	1216	1435	1243
Hiripitiya	482	449	477	461	458	469	510	503	511	437	486	528	939	1375	1616	1100	1171	1189	1378	1579	1543
Maho	427	392	311	330	311	331	365	409	389	411	449	396	668	1180	1616	1191	1623	1716	2012	2201	1817
Makandura	378	360	367	399	364	277	382	292	351	338	334	449	667	987	1337	1235	2072	2177	3191	4278	2709
Mawatagama	555	510	482	401	500	485	530	555	589	471	681	777	1584	2116	2087	1986	2142	2916	3161	3219	2728
Narammala	718	737	635	536	531	493	535	522	510	565	624	1073	2170	2201	2512	2759	4150	4655	5076	4615	4577
Nikaweva	181	162	124	109	124	127	171	223	203	181	204	265	410	772	906	818	619	593	845	1245	1036
Polgahawela	818	830	759	758	743	748	761	780	871	839	1015	1114	3098	2884	3867	3736	4749	5598	5369	5166	5120
Wariyapola	353	374	379	369	327	288	311	393	360	295	335	371	869	1236	1160	1111	1210	2264	2009	1911	1663
Alawwa	342	371	381	400	386	400	461	477	481	953	858	1625	3102	2082	2487	3233	3689	3310	2411	3673	2810
Katupotha	236	257	252	210	108	181	133	152	174	128	207	172	412	405	186	406	752	1180	1052	1709	1795
Hettipola	340	338	208	286	315	232	258	313	308	265	317	241	127	686	850	690	702	398	1849	4340	4235

*Indicates start of the epidemic. Figures underlined denote peak.

with great suddenness, but in the second case the onset of the epidemic would appear to be relatively gradual, although in both cases the hospital attendances were actually trebled on a single day (in one instance on the *first* day of the week, in the other on the *fifth* day).

When the figures given in Table I are read in the light of this explanation, it is difficult to avoid the conclusion that the onset of the epidemic was marked by the sudden and almost simultaneous outbreak of sickness over a wide area.

(2) MODE OF ONSET : PRIMARY INFECTIONS OR RELAPSES.

I now come to the evidence upon which Colonel JAMES bases his contention that the onset of the epidemic was associated with a gradual increase in the number of primary infections.

On the clinical side this evidence comprises the temperature charts of sixty-three patients admitted into the General Hospital, Kandy, between the first week of October, 1934 and the second week of November, the latter being about 2 weeks before the onset of the epidemic in the Kandy district.

In Colonel JAMES'S opinion many of the patients to whom these charts refer were suffering from primary attacks of benign tertian or malignant tertian malaria. So far as species diagnosis is concerned the official report states —and I fully endorse this view—that " the species diagnosis made during the epidemic was not very accurate," and the report goes on to state, in reference to Kandy Hospital, that the partially trained laboratory assistants, who were not working under expert supervision, could not, in February 1935, have spent more than 2 or 3 minutes on each film.

But assuming that the species diagnosis was reasonably accurate, and assuming that many of these charts refer to recent and not to *delayed* primary infections—relapse cases would not usually be admitted as in-patients—this fact, if fact it be, has little bearing upon the point at issue.

It has never been suggested that prior to the outbreak of the epidemic transmission was completely in abeyance. It is known that a considerable percentage of out-door patients are at all times registered as malaria, it is known that the spleen-rate in the Kandy district was of the order of 5 to 20 per cent., the atmospheric conditions were favourable to transmission, and there is reason to believe that a sufficiency of insect-carriers was present in the district.

In these circumstances, the admission of a small number of primary cases —the precise number is not stated—during the 6 weeks preceding the onset of the epidemic to a large central hospital serving a large city and a densely populated tract is not surprising. The relevant point here is whether the sudden outbreak of sickness which commenced in the Kandy district about the first week of December, was due to relapses (which would rarely gain admission to hospital) or to recent primary infections. And on this point these temperature charts, which cover the period from 1st October to 11th November, throw no light.

So much for the clinical evidence. On the parasitological side Colonel JAMES states that the failure of the Pathologist of the Colombo General Hospital to detect gametocytes in 230 blood films of patients admitted to this hospital for malaria between 1st October and 11th November, 1934, is striking evidence that most of the patients were suffering from primary attacks.

These 230 patients include thirty-six residents of Colombo Municipality, and the official report of the Pathologist, Colombo General Hospital, in respect of these cases, states that in eleven films B.T. parasites were found, in eight films M.T. parasites, and in seventeen films (47 per cent.) no parasites were detected.

The high percentage of negative films is noteworthy, and it perhaps suggests that at this time, *i.e.*, before the commencement of the epidemic in Colombo, the infections were extremely light, and the failure to detect gametocytes by means of routine blood examinations conducted for diagnostic purposes cannot, therefore, be regarded as conclusive evidence of their absence.

In further support of his contention that the epidemic started gradually, Colonel JAMES states that the weekly number of positive films detected by the Pathologist, Colombo General Hospital, showed a slow, steady increase from the first week in October, 1934, until about the middle of December, when a sharp rise occurred.

Now the epidemic, so far as sickness is concerned, broke out in Colombo District about 10th November, and Colonel JAMES, in stating that the epidemic started gradually, presumably refers to a gradual increase in the number and intensity of infections during October, November and the early part of December. It is doubtful whether the facts and figures advanced by Colonel James justify this conclusion. In the first place, the figures refer to the result of routine blood examinations conducted for diagnostic purposes and not to accurate quantitative estimations of the number and intensity of infections. Secondly, these figures include patients admitted into the Colombo General Hospital from all parts of Ceylon, including districts in which the epidemic started earlier than in Colombo.

To obviate this difficulty the parasitic findings in respect of the residents of Colombo should surely be considered, and the official figures are given in Table II on the next page.

Colombo (population 244,163) is not ordinarily a malarious locality, but its residents constantly visit endemic and hyper-endemic areas, and it is, therefore, to be expected that a small number of primary cases should be admitted to the General Hospital, Colombo, during every malaria season. Assuming that all the positive films in Table II refer to recent primary infections, it will be seen that the weekly figures do not exhibit any appreciable rise until early in the month of December when a sharp and sudden increase occurred. It is also noteworthy that the outbreak of sickness which took place about 10th November, was not followed by any appreciable increase in the number

<div align="center">TABLE II.</div>

Parasitic Findings in Respect of Residents of Colombo admitted to the General Hospital, Colombo, for Malaria during the period from October 6th, 1934, to December 29th, 1934.

Week Ending.	Number of Positive Films.		Total.
	M.T.	B.T.	
October 6th	0	1	1
„ 13th	2	2	4
„ 20th	0	3	3
„ 27th	0	1	1
November 3rd	3	2	5
„ 10th*	3	2	5
„ 17th	4	5	9
„ 24th	2	1	3
December 1st	5	6	11
„ 8th	8	19	27
„ 15th	42	21	63
„ 22nd	75	16	91
„ 29th	57	12	69

<div align="center">*Commencement of epidemic (morbidity).</div>

of admissions (? primary cases) until approximately 1 month later. What was the cause of the delay of about 1 month between the sudden onset of sickness and the first appreciable increase in the number of admissions ? May it not be that the initial outbreak of sickness (which is ignored by Colonel James) was due to a sudden outbreak of relapses which were of so mild a nature as not to require admission to hospital, whilst the sudden increase in the number of admissions early in December reflects the sudden outburst of primary infections at this period ?

The figures given in Table II refer to those malaria cases in whose blood malaria parasites were detected. The official figures in respect of the total admissions of residents of Colombo for malaria during the months of October, 1934, to March, 1935, are given in Table III.

It will be seen that the number of admissions for malaria during the months of October and November was extremely small, and that in twenty out of twenty-three Divisions of the Municipality the admissions during November showed no increase over the number admitted during the preceding month. On the other hand, a striking increase in the number of admissions occurred suddenly, early in December, so that the total number of admissions during

TABLE III.

ADMISSIONS FOR MALARIA OF RESIDENTS OF COLOMBO MUNICIPALITY, ARRANGED BY DIVISIONS, DURING THE MONTHS OF OCTOBER, 1934, TO MARCH, 1935, INCLUSIVE.

	Maradana.	Dematagoda.	Pettah.	Kotahena.	Slave Island.	Grandpass.	Borella.	Cinnamon Gardens.	Wellawatta.	Mutwal.	Colpetty.	Welikada.	St. Pauls.	Fort.	Bambalapitiya.	San Sebastian.	Maligakanda.	Maligawatta.	Mattakkuliya.	Timbirigasyaya.	Havelock Town.	New Bazaar.	Narahenpitiya.
October ..	3	4	2	1	2	1	3	2	1	1	1	0	0	1	0	0	0	1	0	0	1	0	0
November..	10	5	4	2	3	4	6	0	2	1	1	0	0	0	1	1	0	0	0	0	2	7	0
December	90	40	56	48	19	33	37	24	22	20	20	22	7	8	17	7	16	10	4	5	6	3	7
January ..	125	63	47	47	38	36	35	33	33	29	28	24	14	14	13	13	11	10	8	8	7	6	4
February ..	60	35	40	17	18	18	17	6	23	13	16	2	1	6	6	12	4	5	2	0	2	15	3
March ..	39	22	31	19	6	5	7	7	8	9	11	0	1	1	11	3	1	3	2	2	2	9	0
	327	178	180	134	86	97	105	72	80	82	77	48	23	30	48	30	32	29	16	15	20	40	14

Total .. 1,778

this month was 539 or about eleven times the number admitted during the preceding month.

If, therefore, it is permissible to draw any inferences from the figures referring to the total number of admissions for malaria to the General Hospital, Colombo (residents only), it must surely be that serious cases requiring admission to hospital (? primary infections) commenced to occur suddenly about 1 month after the sudden onset of sickness in the Colombo district.

In these circumstances, it is held that the clinical and parasitological evidence upon which Colonel JAMES relies, imperfect though it may be, not only does not justify the conclusion that the onset of the Ceylon epidemic was associated with a slow, steady increase of primary infections, but it lends support to the hypothesis that the onset of the epidemic was associated with a sudden outbreak of relapses which was followed about 1 month later by a sudden outburst of primary infections.

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STUDIES ON ATEBRIN.[*]

A CONTROLLED FIELD EXPERIMENT TO TEST THE RELAPSE VALUE OF ATEBRIN.

BY

HENRY FOY,

ATHENA KONDI,

AND

MICHAEL PERISTERIS.

During the summer and winter of 1932 an opportunity was afforded to test the efficacy of the then new drug atebrin through the courtesy of the makers who supplied us with a quantity for oral, intravenous and intramuscular use. Our work with atebrin has fallen naturally into field, laboratory and clinical studies ; it is the former with which this paper is mainly concerned. Our laboratory studies have been chiefly directed to ascertaining the blood levels, and rates of excretion in the urine and faeces after graded doses ; as well as the mapping of the distribution of the drug in the cellular elements of the body by means of fluorescence microscope in the ultra-violet light. Our clinical studies were in a large part concerned with the relative efficacy of atebrin compared with quinine in clearing the parasites, and controlling the clinical symptoms ; and also with its use in blackwater fever where quinine was contra-indicated. An account of our laboratory and clinical studies will be published later.

The present paper deals with the parasitic relapse rate after treatment with atebrin. Since our work was done in 1932 a vast literature has grown up on this aspect of the subject, and would appear to render the present paper superfluous. We have, however, decided to publish our results partly to round off our other atebrin work, and also because we attacked this relapse problem in a somewhat different way to that of other workers. Our first object in conducting this relapse experiment was to discover an area sufficiently malarious to give our results an unequivocal meaning. Secondly, since the experiment was to be carried out in the field, we wished to assure ourselves that each individual had taken his dose of the drug and for this purpose we decided to administer the treatment personally to each patient. Lastly, we were anxious to make the blood examination as accurate as possible both as to the presence and type of parasites ; 500 to 800 fields were, therefore, examined on every slide, and any doubtful parasite or body was sent for an opinion to Dr. M. A. Barber.

The area chosen was a village (Samsus) on the western side of Epirus (Greece) on a very malarious plain, about 60 kilometres from the sea. The spleen rate of the village in January, 1933, was 92 per cent. and the parasite rate 39 per cent. The parasite rate was necessarily lower than it would have been if the blood examination had been made at the height of the malaria season, July to September. A blood examination carried out in August, 1932, showed a parasite rate of 58 per cent.

The spleens examined were classified according to HACKETT's notation, and their distribution and that of parasites is given below.

Spleens.			Per cent.	Parasites.			Per cent.
P.	..	.. ..	18	*P. vivax*	..	..	68
I.	..	.. ..	38	*P. falciparum*	.,	..	20
II.	..	.. ..	35	*P. malariae*	..	..	4
III.	..	.. ..	7	Mixed	..	.. ..	7
IV.	..	.. ..	1				

The percentage of *falciparum* is probably lower than it would have been had the examination been made earlier. (September—October.)

In order that our relapse results might not be vitiated by transmission of malaria during the experiment we commenced our work in January, well after the cessation of the transmission season which falls roughly between June and October. Our relapse blood smears were taken during the first week of May before the commencement of transmission, but during the relapse period as far as this latter can be caught.

It will be apparent, then, if after anti-malaria treatment in January bloods become negative, and are found to be positive in early May again, the positiveness of the May bloods can with reasonable accuracy be regarded as relapses, since transmission was at its lowest ebb during the January to May period.

Two hundred people in the village were selected at random (but avoiding children under the age of 5 years on account of the difficulty of getting young children to take drugs easily). These 200 people were divided into four groups of 50. Unavoidable dropping out and uncertainties finally reduced the 200 to 187. Since the blood examination will never reveal 100 per cent. of those harbouring parasites, especially if they are few in number, we decided to take three blood smears from each person in January ; one on each of the 2 days previous to the commencement of the treatment, and one on the first day of treatment, and all persons showing parasites on any of these days were regarded as positive. The same system was followed with the relapse smears taken in May, three smears being taken on alternate days ; in this way we hoped to reduce the margin of error as much as possible.

The types of treatment administered were as follows :—

GROUP				Persons.	GROUP				Persons.
I. Atebrin	..	..	..	49	III. Quinine	..	..	..	48
II. Quinine and plasmoquine					IV. (Control) Bicarbonate of				
simplex ..	..	..	40		soda	..	..	..	50

GROUP I.—ATEBRIN.

The individuals in this group of forty-nine persons consisted of twenty-six negatives and twenty-three positives (seventeen *vivax*; three *falciparum*; one *malariae*; two mixed, *falciparum* and *vivax*). The age distribution was between 5 and 60 years. Atebrin was given for a period of 5 days according to the following age scheme :—

4 to 8 years. 0·2 grammes (= 2 tablets) per day.
Adults. 0·3 grammes (= 3 tablets) per day.

Thus, in the 5 days, the children took 1 gramme, and the adults 1·5 grammes. We believe that this treatment has now become a standard one, but at the time of our experiment there was some uncertainty concerning the optimum dose for children. We are inclined to think that the great percentage of relapses that occur among atebrined children is in part due to the small amounts of atebrin given ; and from work done in the Refugee Hospital in Salonika we would advocate 1·5 grammes being given to children between the ages of 4 to 8 years, spread over a period of 7 days. We have had experience of giving large amounts of atebrin (in one case ten tablets, and in another fifteen tablets taken in error all at one time) without more serious ill-effects than a passing nausea and vomiting. In a later paper we shall show that fluorescence analysis reveals some atebrin deposited in the cells of the liver and spleen and that the quantity found there is in some measure dependent upon the rapidity with which the drug is taken ; it appears that small doses can be dealt with and excreted rapidly, but that larger amounts are eliminated more slowly and accumulate temporarily in the liver and spleen and possibly in the reticulo-endothelial system.* GREEN (1934) has reported all manner of ills resulting from atebrin treatment, but our own experience of treating many hundreds of cases personally is that any toxic effects that follow are generally very mild and we have never encountered anything more serious than vomiting for a short time after atebrin has been taken on an empty stomach. If combined with plasmoquine, sometimes gastric symptoms supervene, due to careless grading of the plasmoquine.

As a result of the treatment all became negative.

Relapse blood smears were taken in May (three smears from each person on alternate days) and among the forty-nine, six were found to be positive (four *vivax*; one *malariae*; one non-classified) giving a relapse rate of 12 per cent.

We decided to take the quinine histories of all our patients during the period of January to May, in order to ascertain whether negatives found in May

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*According to TROPP and WEISE (1933) only a small quantity of atebrin is excreted in the urine unchanged, a large part being destroyed in the body. According to KEHAR (1935), 50 to 70 per cent. of the atebrin administered is excreted in the urine, and is demonstrable up to about 69 days after the last dose. Our own opinion is at the moment that a certain amount is incorporated in the parasites, as can be shown by fluorescence analysis ; a certain amount is deposited temporarily in the cells of the liver and spleen (and perhaps other organs) and skin. So far we have no information as to the time that the drug remains in these organs, or whether it undergoes any change while in the body. We should here like to direct attention to the presence of atebrin in the faeces, and to suggest that this must be taken into account in any discussion of excretion values.

could in any way be regarded as due to reinforcement of our treatment with doses of quinine taken on their own account. From this point of view the atebrin group is very instructive since only one took quinine (20 cg.) during the 4 months that elapsed between January and May: we can assume that such a quantity would have no effect on the relapse smear that we took in May. It might not be unreasonable to assume that as no quinine was taken by this group the need for it was not felt (*i.e.*, that they had no clinical relapses). This last assumption is to some extent vitiated by the habit that the Greeks have of taking quinine for all and every complaint. But the difference between the atebrin group and the other groups with respect to their quinine consumption is suggestive. Regarding the age distribution of those relapsing, numbers are too small to warrant any conclusions being drawn and the same applies to the parasitic distribution of the relapses.

Another striking fact noticed among those taking atebrin was the ease with which they took their treatment, an especially important point when field work or mass treatment is being undertaken. The effective dose of atebrin is non-toxic, and unlike the effective dose of quinine (5 to 15 grammes) gives rise to no unpleasant symptoms, such as dizziness, ringing in the ears, etc. Further, the period of treatment is short (5 to 7 days) and consequently easy to control. In addition, it can be given to pregnant women* as well as to those sensitive to quinine (blackwater fever).

Group II.—Quinine and Plasmoquine simplex.

This group consisted of forty individuals made up of twenty-seven negatives and thirteen positives (nine *vivax* ; two *falciparum* ; two *malariae*) of ages varying between 5 and 60 years. Ten persons in this group refused to continue their treatment complaining of varying degrees of quininism, etc. The drugs were given for a 5 day period according to the following scheme.

5 to 10 years: 0·8 grammes quinine + 0·01 grammes plasmoquine per day.
10 to 15 „   1·5   „   „   + 0·01   „   „   „
   15 + „   2·0   „   „   + 0·02   „   . „   „

The doses decided upon were those which were regarded as likely to give good results without causing too great a number to drop out on account of quininism, a somewhat difficult mean to strike in a field experiment, where individuals were not attending our dispensary because they were clinically ill. Our own feeling is that the amount of quinine given was not quite sufficient to produce the best relapse results, but on the other hand greater doses would have undoubtedly caused more to give up their treatment.

The result of the treatment was that all the positives became negative at the end of the 5 days.

As in the case of the atebrin, relapse blood smears were taken in May (three smears from each person on alternate days). The examination of these smears

*Intravenous atebrin given to cats causes stimulation of the uterine muscles, according to SAPEIKER (1931).

showed that of the forty persons, seven had become positive, a relapse rate of 17·5 per cent. compared with the 12 per cent. in the atebrin group. Further, the number of persons in this group who took quinine between January and May was five, and of these one took it in an amount, and at a time, that would affect our relapse smear (more than 1 gramme, 1 week before we arrived). If this one is assumed to have taken quinine for malaria, and further that he would have been blood positive had he not taken this quinine, we can add a further one to the already existing seven positives, making a total of eight positives out of the forty persons, a relapse rate of 20 per cent.

Group III.—Quinine.

This group was made up of forty-eight individuals of an age distribution similar to that of Groups I and II. Thirty-two of these individuals were negative, and sixteen positive (thirteen *vivax* ; two *falciparum* ; one mixed, *falciparum* and *vivax*). Quinine was given according to the following scheme for 5 days.

5 to 10 years : 0·8 grammes quinine daily.
11 to 15   ,,   1·5   ,,   ,,   ,,
  15 + ,,   2·0   ,,   ,,   ,,

Again the treatment, as was to be expected, resulted in all the positives becoming negative.

Relapse blood smears taken in May, as in the case of Groups I and II showed thirteen positives out of the forty-eight persons ; a relapse rate of 27 per cent.

Quinine histories of this group showed that nine of the group had taken quinine between the January and May period, and two had taken it in quantities and at a time that was likely to effect the result of our blood smear taken in May. (One of them had a 1 gramme injection the day previous to our arrival ; the other had taken 8 grammes during the fifteen days that preceded our May visit.)

If we make the same assumption with regard to these two as in the case of the one in the quinine and plasmoquine group, namely, that they took the quinine for malaria, and that had they not taken it when they did, in the quantity they did, their blood would have been positive, the total number of positives in this group in May is raised to fifteen, giving a relapse rate of 31 per cent., two and a half times as high as in the atebrin group.

Group IV.—Sodium bicarbonate.

It is an advantage if some form of " treatment " is given to the control group, in order to gain their co-operation and attendance for the taking of blood smears.

There were fifty people in the group, made up of twenty-nine negatives and twenty-one positives (eleven *vivax* ; eight *falciparum* ; two mixed, *vivax* and *falciparum*). As a result of the " treatment," the bloods of all the individuals remained as they had been before the commencement of the experiment.

An analysis of this control group is rendered difficult on account of the

facts that (1) some of the original January negatives became positive in May ; (2) some of the original January positives became negative in May, although it was reasonably certain that they took no quinine ; (3) 32 per cent. of the group took quinine in the period of January to May.

A comparison between the January and May positives shows that of the original twenty-one positives in January, six became negative in May, although only one of them took quinine in quantities (16 grammes) and at a time likely to affect our May smear. The remaining five who became negative all took less than 1 gramme in the 4 months intervening between January and May ; either these five then underwent spontaneous cures, or our three smears taken in May failed to reveal a hidden infection.

Regarding the twenty-nine negatives of January : three of them became positive in May, the other twenty-six remained negative. Here again either our January smear failed to reveal a hidden infection, or the three became infected during the winter.

Regarding the percentage in the control group who took quinine, 32 per cent., it will be noticed that this is considerably higher than in either Group I (2 per cent.), Group II (12 per cent.) or Group III (19 per cent.). The infection rate in the control group in May (36 per cent.) remained substantially what is was in January (42 per cent.). Further the general relapse rate in all the treated groups considered as a whole was 19 per cent., whilst in the control group is was 36 per cent., indicating that although a large percentage took quinine they took it in amounts that were ineffective. A comparison of the relapse rates in all the groups are summarised below, the last percentage figure indicating what the relapse rate would have been if those are included who took effective doses of quinine at a time likely to affect our May smear.

Atebrin	12 per cent.	
Quinine and plasmoquine simplex ..	17 to 20	,,
Quinine	27 to 31	,,
Control	36 to 42	,,

SUMMARY.

A field experiment was carried out to test the relapse value of atebrin. A group of 200 persons were divided into four groups : Group I was given atebrin for 5 days in doses according to age ; Group II was given quinine and plasmoquine simplex for 5 days ; Group II had quinine only. A fourth group was used as a control. The experiment was conducted in a transmission-free period of the year, the relapse rates in the various groups being those shown in the table just above.

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A PRELIMINARY NOTE ON THE LIFE HISTORY OF *SCHISTOSOMA TURKESTANICUM* SKRJABIN, 1913.

BY

C. MACHATTIE, F.R.C.V.S., D.V.S.M.,*

Veterinary Vaccine, Serum and Lymph Institute, Iraq.

Schistosoma turkestanicum was first described by SKRJABIN (1913) from the branches of the portal vein in local cattle (*Bos taurus*) of the Antic-Ala in the Syr-Darja District of Russian Turkestan.

MAROTEL (1908) reported the occurrence in France, of a schistosome in a home bred cow which he considered to be identical with *S. bomfordi* Montgomery, 1906. PRICE (1929), however, points out that the egg of MAROTEL's schistosome had two spines, one at each end, and the number of testes was sixty. As *S. turkestanicum* is the only schistosome reported from cattle possessing a large number of testes and an egg with a prolongation at each end, it appears probable that MAROTEL was actually dealing with this species and not *S. bomfordi*. POPOV (1926) recorded the presence of *S. turkestanicum* in a cat in Kazakstan. YAMAGIWA (1931) described the lesions observed in thirty-three cases in Mongolian cattle, caused by the presence of *S. turkestanicum*. Adult worms were detected in the portal system chiefly in mesenteric veins. He found that the parasite was pathogenic to cattle but not to such an extent as *S. japonicum*. He attributes such differences in pathogenicity between these two species to the decided differences in the number of eggs laid by them.

MacHattie and Chadwick (1932) reported the occurrence of *S. turkestanicum* in Iraq, and Bhalerao (1932) described the morphology of specimens

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MACHATTIE and CHADWICK (1932) reported the occurrence of *S. turkestanicum* in Iraq, and BHALERAO (1932) described the morphology of specimens

sent to him by the writer. PRICE (1929) transferred *S. turkestanicum* to the genus *Ornithobilharzia* Odhner, 1912, on the basis of morphological similarity to other species of that genus. PRICE writes, " The fact that both *O. bomfordi* and *O. turkestanicum* are at present known only from mammalian hosts does not appeal to the writer as being a matter of sufficient importance to justify their retention in the genus *Schistosoma*; in view of the morphological relationship of *O. bomfordi* and *O. turkestanicum* to species occurring in birds, it may be assumed that these parasites, which are of rare occurrence in their mammalian hosts, may be only accidental and facultative parasites of these hosts, and it may be surmised that they are normal parasites in birds of some sort."

The life history is unknown not only of *S. turkestanicum*, but of all the species of the genus *Ornithobilharzia*.

Distribution in Iraq.

Far from being of rare occurrence in domestic animals in Iraq, *S. turkestanicum* has been found to constitute a serious menace to stock in the southern areas. The parasite can be demonstrated daily in thousands in the slaughterhouses at Amarah, Diwaniyah, Nasiriyah, Kerbela, Hilla, Nejaf and Khanaquin or in any flock visited between Amarah and Basrah, by making a postmortem of the most debilitated sheep or goat. It is, however, in the tribes popularly known as Marsh-Arab and in the rice field areas between Amarah and Basrah that the really formidable infestations occur. Between these towns there are immense areas of permanent shallow water on either side of the Tigris, generally some distance from the river. Not a single specimen of *S. turkestanicum* has been found in Northern Iraq and Iraq Kurdestan.

In the Amarah-Basrah areas about 80 per cent. of sheep, goats, cattle and water buffaloes are infected and about 15 per cent. of horses, donkeys, mules and camels. Man, however, appears to be immune. Repeated examinations of human faeces and urine have been entirely negative, yet the snail host is regarded as edible by Marsh-Arabs. Several pools known to be heavily infested are regularly used by the tribespeople for drinking and ablution. Twenty-nine cats and 256 dogs destroyed at Amarah in accordance with anti-rabies measures were examined with negative results. Eleven domestic ducks and three tame geese which had reached maturity and moved daily in water regularly used by heavily infected sheep showed no trace of infection.

The following wild birds were shot near infected pools :—

Two grey lag goose (*Anser anser*).
One common shelduck (*Tadorna tadorna* L.).
Two pintail (*Anas acuta* L.).
Two Jack snipe [*Lymnocryptes minimus* (Brünn)].

One white fronted goose (*Anser albifrons* Scop.).
Three mallard (*Anas platyrhynchos* L.).
One common heron (*Ardea cinerea* L.)
One white tailed lapwing (*Chettusia leucura*).

PLATE I.
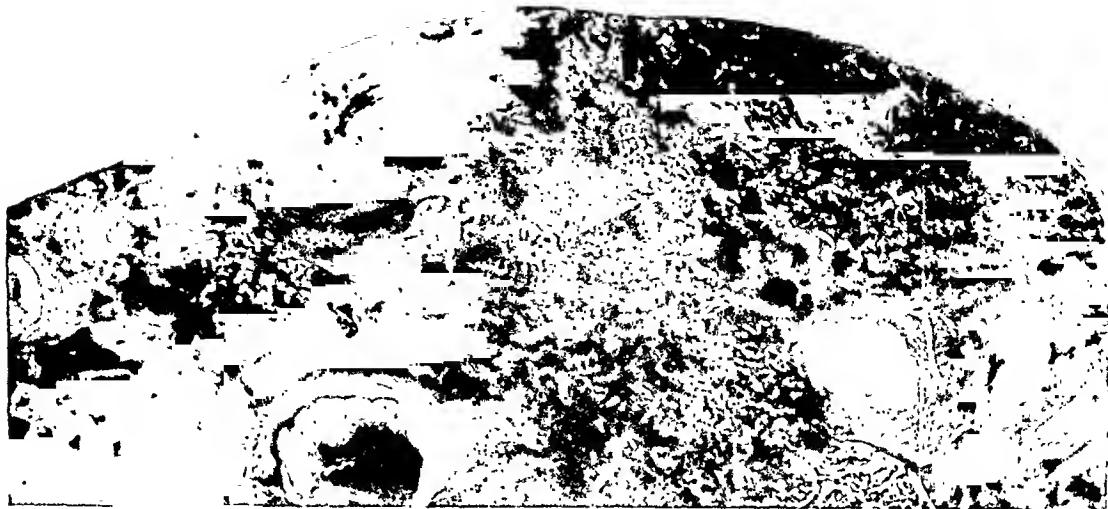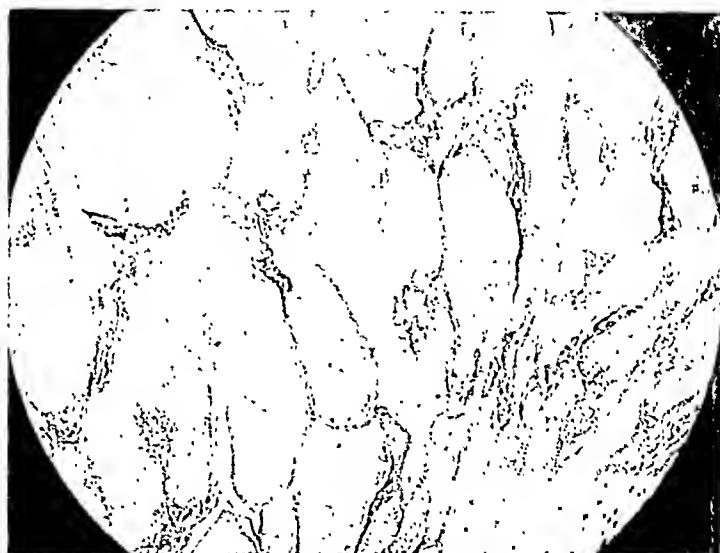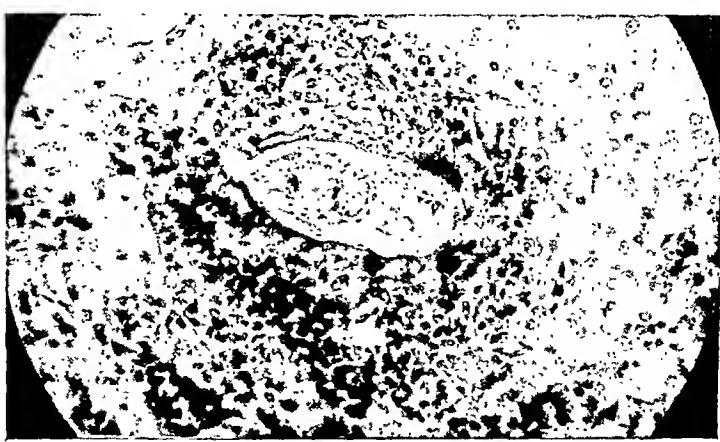
FIG. 1.

FIG. 2.

FIG. 3.

FIG. 1.—Cross section of a goat's liver showing thickening of vessels.
FIG. 2.—Advanced cirrhosis of liver in a sheep, directly and indirectly due to

FIG. 4.
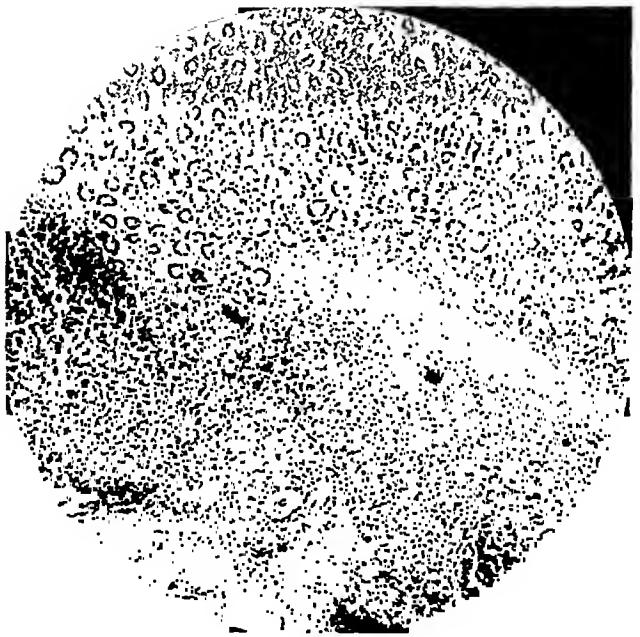
FIG. 5.

FIG. 4.—Longitudinal section of a mesenteric blood vessel (sheep), showing large numbers of the parasite.

FIG. 5.—Nodules in the intestinal sub-mucosa (sheep).

Fig. 6.
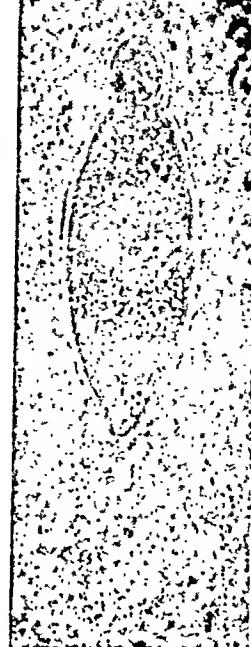
Fig. 7.

Fig. 8.
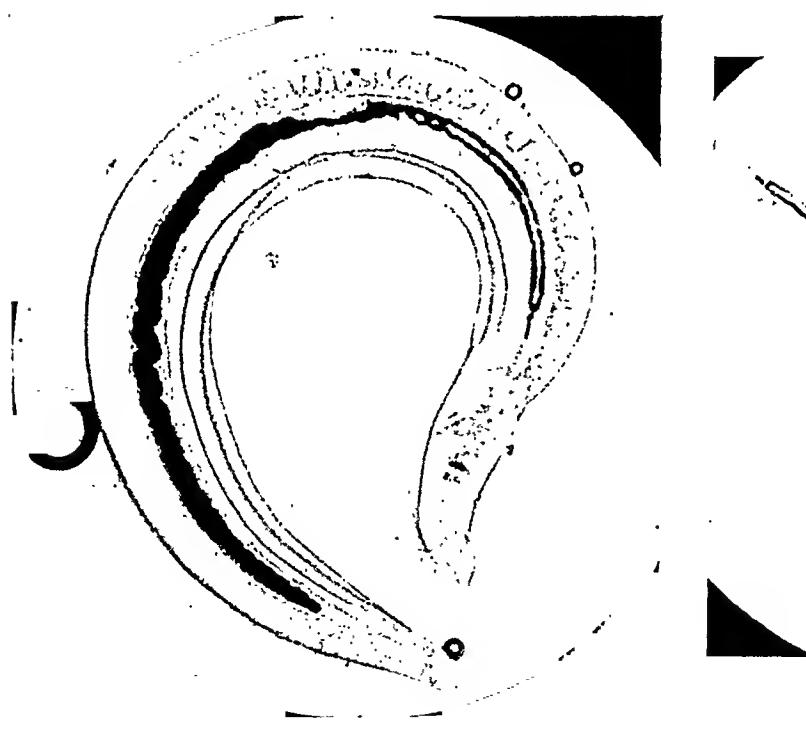
Fig. 9.

Fig. 10.

Fig. 6.—Mature and immature eggs as shown in scrapings from the mucous membrane of the intestine of a sheep.

Fig. 7.—Immature egg.

Fig. 8.—Mature egg.

Fig. 9.—Male and female.

Fig. 10.—Free female.

of origin of good and bad casings corresponds to the localisation of schisto-somiasis. The injury to the wall of the intestinal tract caused by the deposition of spiny schistosome eggs and reactionary tissue changes, is such that patchy thickening of the intestine is produced.

Proper nutrition of the animal body becomes, with age, a matter of increasing difficulty and the fattening of sheep and goats an impossibility. The total economic loss is great, and is not so much the result of occasional deaths directly due to the parasites, but to the weakening of the animal's constitution which predisposes to deaths on a large scale when periods of drought and food shortage occur.

Morphology.

The Iraq schistosome (Plate Figs. 9 and 10) both in its natural state and artificially bred in experimental animals, would appear to be identical with *S. turkestanicum* SKRJABIN, 1913. During the past 3 years the writer has made a very careful comparison of both smooth cuticled specimens and the tuber-culated types referred to by BHALERAO (1932)* and has formed the opinion that the false tubercles form the only difference between the types, the tuber-culated appearance being due to contraction of the cuticle of the male worm, an appearance only observed when the worm is not killed quickly. The maximum and minimum total length measurements of specimens, from a collection of over 10,000 are : Male, 2 to 10 mm. ; Female, 2 to 8 mm.

Specimens from sheep and horses were submitted to Professor LEIPER and Dr. H. A. BAYLIS, who identified them as *S. turkestanicum.*

Natural Infection in the Mollusc Host.

Since *S. turkestanicum* occurred in almost every sheep and goat in the Amarah Marsh-Arab and rice field areas, it followed that the snail host must be a very widespread and prevalent one in that district. Investigations were carried out by the writer in June and December, 1931, June and September, 1932, and May and September, 1933. The infected pools were traced from infected sheep slaughtered at Amarah slaughter-house and the site of infection confirmed by the purchase and postmortem examination of sheep at the water pools so traced.

A thorough investigation of the molluscan fauna was undertaken, and revealed that the most prevalent snail in water used by infected sheep and goats was *Lymnaea tenera euphratica* Mousson.

The other species found were *Bulinus truncatus* Aud., *Corbicula fluminalis* Mull., *Lymnaea canalifera* Mouss., *Melanoides tuberculata* Mull., *Melanopsis nodosa* Fer., *Planorbis philippii* Monts.

In the Baghdad area where infections of *S. turkestanicum* are comparatively

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* All of BHALERAO's specimens of *S. turkestanicum* were collected and preserved by the writer. So far as is known, the worm does not occur in India.

rare, the only host of mammalian schistosome cercariae is *Bulinus truncatus*, which is infected with both *S. haematobium* and *S. bovis*; (*S. mansoni* does not occur in Iraq). It was, therefore, of interest to find that in the Amarah area this species of snail was comparatively rare, about one *Bulinus* being found to every 500 *Lymnaea*.

The snails were subsequently examined for larval trematodes. All species other than furcocercous cercariae were considered irrelevant to the present investigation. One specimen of *Bulinus truncatus* was infected with a brevifurcate apharyngeal distome. A few specimens of *Melanoides tuberculata* were found heavily infected with a pharyngeal longifurcate distome with eyespots. *Lymnaea tenera euphratica* harboured two species of furcocercous cercariae. An apharyngeal longifurcate distome occurred in 0·7 per cent. of the snails, and an apharyngeal brevifurcate distome was present in 1·8 per cent. of the snails.

Experimental Infection of Laboratory Animals.

The apharyngeal brevifurcate distome cercariae from *Lymnaea tenera euphratica* were fed to laboratory reared animals. *S. turkestanicum* was recovered from the mesenteric vessels and liver. Details of these experiments are given below.

The cercariae from *Bulinus* were also pipetted into the mouth of a white mouse, which subsequently developed a *S. haematobium* infection. The apharyngeal longifurcate distome cercariae from *Lymnaea tenera euphratica* and the pharyngeal longifurcate cercariae from *Melanoides* were likewise fed to laboratory reared rabbits and white mice. These animals were killed 3 months after exposure to infection, but no schistosomes were found. This result was to be expected as the cercariae in question displayed characters unlike those of any known mammalian schistosome. However, the morphology of adult *S. turkestanicum* differs so markedly from that of other members of the genus (excluding *S. bomfordi*) that it seemed quite possible that its cercariae might also prove an aberrant form. It was, therefore, considered safer to expose animals to infection from all the species of furcocercous cercariae recovered from the snails.

Details of the Experiments.

In May, 1933, about 4,000 *L. tenera euphratica* were collected and examined at Amarah and the products of 76 snails infected with the cercariae of *S. turkestanicum* were pipetted into the mouths of eight white mice, six rabbits and five guineapigs, all bred in the laboratory. Each animal received at least three heavy doses on each of three successive days (9th, 10th and 11th May). Mice showed signs of irritation in the mouth and repeatedly rubbed the mouth with the feet, but no mouth sores were ever detected as the result of passage of cercariae.

The animals were taken to Baghdad where after 2 months the faeces were examined daily for eggs, but with entirely negative results. After 3 months the animals were killed, the following results being obtained :—

Mouse 1.—Killed. Three female *S. turkestanicum* were washed out from the mesenteric vessels.

Rabbit 1.—Killed. Eleven female *S. turkestanicum*, six of which showed a rudimentary egg in the uterus, were washed from the mesenteric vessels.

Rabbit 2.—Sixteen *S. turkestanicum*, again all females, were recovered from the mesenteric vessels.

Guineapig 1.—Entirely negative.

Mouse 3.—Killed. From one half of the liver, four males and females in copula were recovered, in two cases the female was lying the wrong way round in the canal. In addition two free males and one free female were recovered from the liver. Only one female was washed from the mesenteric blood vessels.

In all the above animals no parasites or eggs were found in the lungs, bladder wall, centrifuged urine, preparations of faeces and smears from the intestinal wall.

Experimental Infection of Mollusc Host.

In order to obtain a supply of uninfected *L. tenera euphratica* a collection was made from the Baghdad ditches. (It has already been pointed out that infection of sheep with *S. turkestanicum* is rare in this district). Numbers of these snails were crushed, but no furcocercous distomes were found and a batch kept in the laboratory for 6 weeks under close observation also proved to be uninfected.

Three emaciated sheep from the Chiyala area of Amarah, all heavily infected with *S. turkestanicum*, were sent to the laboratory at Baghdad. On postmortem, scrapings from the mucous membrane of the intestine showed numerous *S. turkestanicum* eggs with and without a developed miracidium. Scrapings rich in eggs and also some passed faeces were added to a jar containing the uninfected *L. tenera euphratica* from Baghdad. The snails readily fed both on the mucous membrane and the faeces. Eighteen days later one of the crushed snails showed a heavy infestation of the liver with mammalian schistosome cercariae. These were morphologically identical with the cercariae found in *L. tenera euphratica* from the pools used by heavily infected sheep at Amarah. Cercariae emerged from three other snails on the twenty-first day, and sixteen out of twenty snails proved to be infected on crushing.

Batches of *Bulinus truncatus*, *Melanoides* sp. and *Melanopsis* sp. collected from Baghdad were exposed in a similar manner to infection with the miracidia of *S. turkestanicum*. Three weeks later, the snails were crushed and dissected but no trace of sporocysts or cercariae were found.

*Characters of the Mollusc Host.**

Shells of snails infected with *S. turkestanicum* (Plate Fig. 11) were sent to Mr. G. C. ROBSON, of the British Museum (Natural History) who submitted them to Major M. CONNOLLY. He identified them as *Lymnaea tenera euphratica* Mousson, " phase " *Angustior* Mousson.†

* No trace of *Barrouxia* parasitation has been recorded in Iraq, either in the case of *Lymnaea* or *Bulinus* species.

† From specimens of *Lymnaea tenera euphratica*, supplied to her by the writer, MARIAN ROTHSCHILD (1936) has described the process of encystment of a cercaria belonging to the heterogeneous Gymnocephalic group.

Both in the natural infections at Amarah and in the experimental infections at Baghdad, the infected *Lymnaea* is always of small size, approximately 12 × 6 mm. Large specimens were never found infected. The " phase " therefore appears to have some significance, and in the writer's experience *L. tenera euphratica* Mousson " phase " *Angustior* Mousson is the only host of the cercaria of *S. turkestanicum*.

The pathological effect on the snail is very marked, although no lightening in colour of the shell occurs, as in the case of *Bulinus truncatus* infected with *S. haematobium* or *S. bovis*. Infected snails placed in tubes never lived more than 8 hours and, unlike uninfected specimens, rarely survived transport from Amarah to Baghdad.

Iraq experiences two extremes of climate during the year. May to October is very hot and dry, and, in sharp contrast to this, December to April is cold and wet. With the onset of the cold weather the snail hosts of *S. turkestanicum*, *S. haematobium*, and *S. bovis* die in thousands. In the rare specimens of *Lymnaea* then found infected, the cercariae are moribund and incapable of effecting penetration. Thus it would appear that stock can drink from infected pools during the winter with comparative impunity.

L. tenera euphratica is common along the banks of the lower Euphrates, but the most northerly record appears to be 5 miles north of Samara (ANNANDALE and PRASHAD, 1919). Thus the distribution of *S. turkestanicum* would appear to be closely linked with that of the snail hosts. Other factors, however, such as suitability of environment, probably play an equally important part.

Development in the Mollusc Host.

The Miracidium.—The surface of the body is covered with long cilia except at the protuberant anterior extremity. Two pairs of cephalic glands with large nuclei are present. A portion of the intestine can be traced through the anterior half of the body. When placed in water, the eggshell swells and two large globules of fluid make their appearance one at the blunt pole and one laterally. By means of the cilia the miracidium rotates the lateral globule of fluid. Pressure is thus increased and probably facilities escape from the egg capsule.

The average measurements of thirty mature eggs containing miracidia were 0·152 mm. × 0·054 mm. (including the posterior blunt process and anterior spine process). The length of the miracidium within the egg was 0·108 mm. × 0·044 mm.

The Sporocyst.—The miracidium develops into a mother sporocyst, giving rise to daughter sporocysts. Eventually the entire liver of the snails becomes permeated. (Plate Fig. 12). No movement of sporocysts was observed.

The Cercaria (Plate Figs. 13 and 14).—Free swimming cercariae were examined alive and dead in saline and in horse serum as recommended by ARCHIBALD and MARSHALL (1931). Mounted specimens were prepared according to these authors' lacto-phenol-gum technique. Carmine, neutral red · and

Delafield's haematoxylin were used for intra-vitam staining. Measurements were taken of free swimming forms, killed by heat according to MANSON-BAHR and HAMILTON FAIRLEY'S (1920) technique. It has already been stated that the cercaria of *S. turkestanicum* is a non-eyed apharyngeal brevifurcate distome. The oral sucker or anterior organ is well developed, but the ventral sucker, situated near the posterior end of the body, is only moderately developed. The body, including the surfaces of both suckers, together with the tail and furcae are covered with fine spines. These are most conspicuous on the tail and furcae. The cuticle is delicate. The penetration glands are variable in shape and position, but there are apparently four pairs of large glands. In the living cercariae the glands appear refractile and glassy but in stained sections they show a finely granular structure. Two groups each of four ducts run forward along the sides of the oral sucker. At the anterior end of the oral sucker are eight piercing spines, four on each side of the middle line. The glands produce a fluid which often adheres to the spines at the exit of the ducts, and this makes them difficult to distinguish. A bladder can be seen at the posterior end of the body, but the writer found it impossible to distinguish collecting tubes and flame cells. An excretory duct runs throughout the tail and furcal rami. The tail contains several cells with clear cytoplasm and large nuclei distributed in the parenchymatous tissue.

Measurements of the cercaria in millimetres.—(Average of 30 specimens.)

Body	$0 \cdot 178 \times 0 \cdot 0620$ mm.
Tail	$0 \cdot 187 \times 0 \cdot 226$,,
Furcae	$0 \cdot 045 \times 0 \cdot 013$,,
Protruded anterior organ	$0 \cdot 019 \times 0 \cdot 0189$,,
Depth of oral sucker	$0 \cdot 058 \times 0 \cdot 022$,,
Diameter of ventral sucker	$0 \cdot 017$

Behaviour of the Cercariae.—The cercariae are discharged from the snail in puffs of about twenty to fifty, at a temperature varying from 20° to 26° C. Emissions can take place either by day or night, but are most rapid in direct sunlight.

The cercariae did not show the predilection for the sub-surface position in a test tube shown by the cercariae of *S. haematobium* but were fairly evenly distributed throughout the water. When tubes of free swimming cercariae were covered with black paper, into which a small window was cut there was no noticeable movement towards the light. In the dark, however, many cercariae attached themselves to the sides of the tube, apparently by the oral and not the ventral sucker. In a petri dish of water, under the dissecting microscope it was observed *that the cercariae always progress body-first.* The most characteristic movement is as follows : The tail bends about its middle and the furcae bend

round to touch the oral sucker. This movement is made slowly and deliberately four or five times, as if the cercaria was preparing itself for the next movement which is actually the same looping movement carried out very much more quickly. The movement is always in a semi-circular direction. The cercariae often rest on the bottom of the petri dish as if they were standing on the furcae. Generally in a test tube when at rest they hang in the water with the head downwards. When attached to a foreign body they often lie quiescent and the only movement is a slow writhing of the furcae which can apparently be moved quite independently of movement of the tail stem. Often a cercaria turns a half somersault, quickly attaches itself by its sucker, and again comes to rest. The extrusion of the ventral sucker, so marked in the cercariae of *S. haematobium* is very rarely observed. There is a very definite protrusion of the anterior organ so that living cercariae observed on a slide often appear as if the body were divided into two parts by a constriction.

When the cercaria is trying to progress under a coverslip, the anterior organ is thrust forward and the anterior sucker attaches itself ; then, accompanied by wriggling of the tail and furcae, the body contracts and is drawn forward. On a slide, body and tail readily separate.

The internal structures of the cercaria are much more difficult to observe and distinguish than are those of *S. haematobium* or *S. bovis*, while considerable difficulty was experienced in watching the movements and behaviour of the free swimming cercariae.

CONCLUSIONS.

1. *Schistosoma turkestanicum* is a formidable and dangerous parasite of domestic animals in the Marsh-Arab and rice field areas of Iraq.

2. In addition to cattle, sheep, goats, and water buffaloes, it has been proved that camels, horses, donkeys and mules are also hosts.

3. The arrest of the egg in the intestinal mucosa and the consequent formation of nodules largely destroys the value of the intestine as a commercial product.

4. Man and domestic and wild birds are apparently immune to infection despite continuous exposure to the attacks of the cercariae of *S. turkestanicum*.

5. The snail host of *S. turkestanicum* is *Lymnaea tenera euphratica* Mousson (" phase " *Angustior* Mousson).

6. Cercariae of *S. turkestanicum* become very sluggish towards the end of the hot season (late September) and disappear from October to May. It seems probable that live stock can then drink from infected ponds with comparative impunity.

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THE EARLY MIGRATIONS OF *WUCHERERIA BANCROFTI* IN *CULEX FATIGANS*.

BY

FRANCIS W. O'CONNOR, M.R.C.S.,[*]

AND

HARRY BEATTY.

*From the Department of Medicine, Presbyterian Hospital, Columbia University,
New York City, U.S.A.*

———————

On the island of St. Croix, Virgin Islands, during dissections of wild *Culex fatigans*, we noticed on several occasions, that larval filariae were present in lesser or greater numbers in fragments of a tubular structure, identified as the anterior part of the midgut. Since, with further dissections, this observation was repeatedly made, we began to appreciate the possible significance of the phenomenon and decided to study it in insects bred and fed under experimental conditions.

At the peak of the periodicity curve of our volunteer, 273 microfilariae were found in 20 c.mm. of blood. On successive nights, thirty to fifty 48 hour-old imagines of *Culex fatigans* were placed in the mosquito net of the volunteer between 10 and 11 p.m., and those which had fed were collected after an hour. Dissections of the recently fed insects were made immediately, and thereafter at regular hourly intervals for 24 hours. Our technique was as follows :—

After stunning the insect with tobacco smoke and removing its legs and wings, the body was placed on a microscope slide in a drop of normal saline. The whole of the rectum, stomach full of blood, the anterior part of the midgut with the oesophageal valve (proventriculus) and oesophageal diverticula were removed intact. Less frequently we were successful in removing the oesophagus with these structures intact as well. After removal, the alimentary tract was then drawn with the dissecting needles into a straight

position and was available for study under the two-thirds objective with binocular microscope. In insects examined immediately after collection, no parasites were observed outside the unruptured stomach, but within less than an hour a few parasites were seen there, having apparently penetrated the wall of the viscus. During the next hour they appeared in increasing numbers and after 2 hours, in newly dissected insects, the first young larvae were found in the thorax. Up to this time and as late as 10 hours after they were collected no parasites were seen in the narrow anterior portion of the midgut, in which, however, persistalsis and reversed persistalsis were usually observed.

After 10 hours exsheathed larvae appeared in increasing numbers at the anterior end of the stomach and shortly afterwards entered the narrow anterior part of the midgut (Fig. 1). Thereafter they were to be seen wriggling sometimes in dense masses and occupying the whole length of the viscus as far as the oesophageal valve or proventriculus. Parasites were not observed to pass this barrier but frequently they were present in the largest numbers immediately below it. (Fig. 2.) While watching the movements of the parasites within the midgut, penetration of the wall was observed by us on two occasions. It would seem then that the concentration of parasites in this narrow channel is adapted to insure access to the thorax by a shorter and less difficult route than is afforded by penetration of the wall of the stomach. These larvae were always very active. Since these experiments were completed the same observations have been frequently confirmed by dissection of recently-fed wild insects collected from houses known to harbour persons with microfilariae in the blood.

To demonstrate these facts in permanent preparations the following technique is that used by us. The dismembered insect is placed on a slide in a drop of normal saline. From the lower end of the abdomen, with dental broaches as dissecting needles, the engorged stomach is gently pressed towards the thorax. This allows greater freedom in making an incision on each side of the posterior segments of the abdomen. One needle is then placed on the last abdominal segments while the other needle holds the thorax in position, and by steady, gentle traction, the rectum, the stomach as well as the anterior midgut with the proventriculus and diverticula of the thorax are abstracted intact. When the presence of parasites in the midgut is recognized under the microscope, the saline on the slide is removed with lens paper and the drying process is allowed to proceed. Any attempt to perforate the stomach at this time causes extravasation of blood and loss of parasites. It is better to wait until the stomach *begins* to show signs of drying, when one or two minute perforations of the posterior part of the stomach are made with the points of the needles. If one waits until the stomach dries or is nearly dry any attempts at·puncture of the now brittle structures causes fracture, especially at the junction of the stomach and anterior part of the midgut. The drying process is allowed to progress and when completed, the slide with the organs
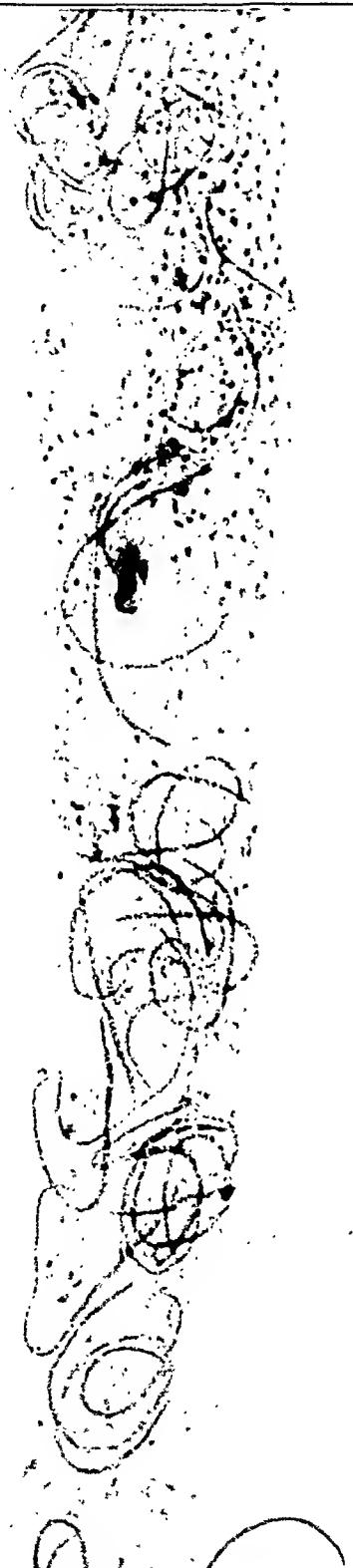
FIG. 1.

FIG. 2.

Fig. 1.—Larvae of *Wuchereria bancrofti* about the middle of the anterior and tubular part of the midgut of *Culex fatigans*, some 13 hours after the infective feed. × 156.

Fig. 2.—Larvae of *W. bancrofti* in masses at the anterior end of the anterior and tubular part of the midgut of *C. fatigans*, some 16 hours after a single infective blood feed.
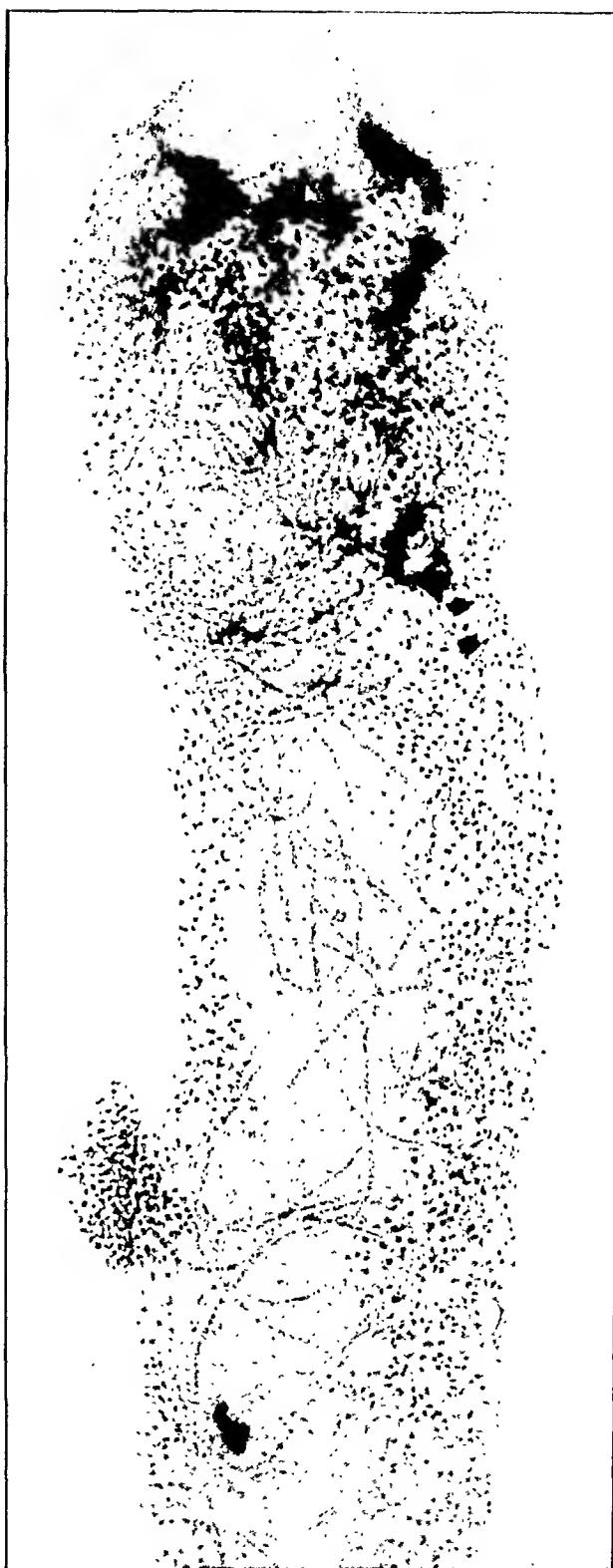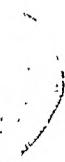
uppermost is placed in a petri dish of tap water. Dehaemoglobinization of the stomach now takes place through the puncture made with the needles. If such punctures have not been made the stomach simply swells, bursts and all its contents are extravasated. After dehaemoglobinization is completed, the specimen is allowed to dry and is then fixed in equal parts of absolute alcohol and ether for half an hour. When the tissues have been dried again they are stained with Bullard's haematoxylin for 10 minutes. The stain is then flushed off and the specimen is blued for 7 minutes in tapwater in a petri dish. The specimen is again finally dried and after clearing in xylol for half an hour, is mounted with Canada balsam under a No. 1 coverslip.

SUMMARY.

1. Within 45 minutes after *Culex fatigans* feeds on a person whose blood contains *Microfilaria bancrofti*, exsheathed larvae are seen outside the stomach, having apparently passed through the undamaged wall of the viscus to enter the abdominal cavity.

2. In St. Croix within 2 hours after such a feed newly arrived larvae are found in the thorax of the insect.

3. Between 10 and 18 hours, possibly longer or shorter according to climatic conditions, larvae mass in large numbers and are found to be very active in the anterior part of the midgut of the insect. Reversed persistalsis of the viscus may or may not play a part in the movements of the parasites along this structure.

4. The penetration by the parasites through the walls of the midgut to the exterior has been observed.

5. Thus exsheathed *W. bancrofti* larvae from the stomach of *Culex fatigans* may reach the thorax by the abdominal route or later, and probably in larger numbers, by the route of the anterior midgut.

6. By modifying well known methods of dissection and staining, a technique has been devised whereby permanent preparations may be made which illustrate some of the foregoing facts.

CORRESPONDENCE.

LYMPHOSTATIC VERRUCOSIS.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

DEAR SIR,

With reference to Dr. L. J. A. LOEWENTHAL's letter on the above subject (TRANSACTIONS, 1936, xxix, No. 5, 558), I wish to say that it was far from my intention to question the validity of his suggested name of " lymphostatic verrucosis " for the disease which he has so very exhaustively described from Uganda.

My own case was described as an example of this disease as seen in Nigeria, and I regret I did not perhaps make it clear that the term " elephantiasis verrucosa " was meant to refer to the disease as exemplified by this particular case, which was definitely associated with elephantiasis.

I described the case, however, under the title, and as one example, of " lymphostatic verrucosis," and there can be no doubt that this name, suggested by Dr. LOEWENTHAL, is the more comprehensive term.

Owerri, Nigeria.
    6th *April*, 1936.

        I am, etc.,
        S. L. A. MANUWA.

ANTELOPES AS RESERVOIRS OF *TRYPANOSOMA GAMBIENSE.*

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

DEAR SIR,

Re Dr. CORSON's letter in these TRANSACTIONS, 1936, Vol. xxix (6), p. 690.

The validity of the experiments carried out in Uganda by BRUCE and his collaborators on the antelope reservoir for *T. gambiense* forms part of the discussion in a paper by me shortly to appear in *Parasitology*. Although not personally associated with Sir DAVID BRUCE at Mpumu, on my arrival in Uganda, in 1910, I joined one of his collaborators, A. D. FRASER, then Capt., R.A.M.C., and some months later took over the laboratory and with it the antelope infected by the Commission. Indeed, it is I, and not the Commission, who must be held responsible for the final conclusions referred to by Dr. CORSON. I doubt,

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therefore, if anyone is in a better position to supplement the information given by the Commission in their report.

The results of my examination satisfied me that there was no reason whatever to doubt the correctness of the earlier experiments, and that the investigation as a whole showed that *T. gambiense* may survive for a long period in antelope. More than that cannot be claimed.

For many years now I have recognised the fallacy of assuming, without proof, that the lake shore trypanosomes of Victoria Nyanza were of necessity *T. gambiense*—an assumption that was sometimes made in the early days. That this fallacy did not vitiate the Commission's reservoir experiments is shown clearly in their report. The fact that all possible precautions were taken against casual infection at Mpumu, including the permanent use of a fly-proof kraal for the antelope and the employment of animal controls, in my opinion justifies the assumption that the trypanosomes eventually recovered from the antelope were indeed the descendants of those originally introduced by the Commission.

The last paragraph of the letter seems to imply that Dr. CORSON believes that the inoculation into man of the trypanosomes recovered from the Mpumu antelope would have settled their identity or otherwise with *T. gambiense*. With this assumption I find myself at variance : it rests on two others, to my mind at present equally unjustified, namely, that *T. gambiense*—or for that matter *T. rhodesiense*—always remains pathogenic to man, whatever the environment in which it lives, and, secondly, that *T. brucei* never becomes pathogenic to man.

Certainly the results of inoculation of the Mpumu antelope strains into man would have been interesting ; but if, as I believe, the investigation as it stands shows that *T. gambiense* can survive for long periods in antelope, an exceedingly important point has been established.

Recent work in Uganda has shown that man's trypanosomes may in certain circumstances when maintained away from man lose the power of infecting him. It has also been shown that strains differ in their pathogenicity to man, and people in their susceptibility to trypanosomes. A " negative " inoculation into man would not have proved that the Mpumu trypanosomes were not *T. gambiense*, or even that that particular strain was non-pathogenic to man. It certainly would not justify the condemnation of the experiment as " contaminated " with *T. brucei*.

Dr. CORSON's view seems to be that if a trypanosome recovered, say from an antelope, is proved to be pathogenic to (? one) man, it is either *T. gambiense* or *T. rhodesiense* ; and that, conversely, if it is non-pathogenic to (? one) man it must, *ipso facto*, be *T. brucei*. This attitude seems to me likely to prejudice the course of further investigations into this problem, for surely it precludes the recognition of changes that may occur in the physiology of a trypanosome.

To one who holds this view the recent Uganda findings will presumably

be attributed to a disastrous (and undetected) series of accidental introductions of *T. brucei*—an interpretation unsupported by evidence and contrary to the results obtained by a careful system of controls. After all, the conclusions reached violate no recognised law of nature.

If a " human " trypanosome can lose touch with man it appears to me not impossible that the converse may take place, namely, that a " non-human " trypanosome may acquire the power of infecting man. It is true that this has not yet been witnessed, but changes of a similar nature have been recorded for other species of mammalian trypanosomes.

It would, of course, be interesting to repeat the Commission's experiments. But even if the results of such a repetition were different, that fact would not invalidate the earlier work. Strains of *T. gambiense* differ from one another, and it may well be difficult to find again strains similar to those obtained by BRUCE and his colleagues at the end of a tremendous epidemic of several years' duration. Moreover, it would be difficult to secure " stricter conditions." Casual infection by tsetse was excluded at Mpumu and interference by other biting flies was reduced to a minimum. The antelope never left their fly-proof kraal, and the only possible " accident " under these conditions would be direct transference of trypanosomes from one animal to another, by insects other than *Glossina* or by coitus. Even if an accident of this kind did occur, it would not seriously affect the conclusions.

My own attempts at repetition will be published shortly. The sooner others are stimulated to further attempts, the better for the administrators of sleeping sickness territories.

I am sure that much confusion has resulted from the undue importance that has been attached to the power of a trypanosome to infect man, and the time has surely come to maintain an open mind both about the stability and the zoological significance of this particular attribute of a trypanosome.

Bournemouth.   I am, etc.,
      24th April, 1936.   H. LYNDHURST DUKE.

CLASSIFICATION OF TYPHUS FEVERS.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

On reading in the TRANSACTIONS (Vol. xxix, No. 6, 561-582) the report of the Ordinary Meeting held on the 20th February, 1936, I observe one or two points, relating to the provisional scheme for the classification of typhus fever we are attempting to follow in the Army in India, on which I should like to comment.

The table reproduced on page 575 has been headed " A suggested classification." This heading does not occur in the original (BOYD, 1935) and is liable to create a false impression, as the table is not a " suggested classification," but is an analysis, according to serological findings, of cases diagnosed typhus fever in the Army in India in 1934.

Based on this analysis, a provisional classification is suggested which does not follow the columns of the table, and which more or less corresponds with the findings in other parts of the world. The suggested sub-groups are as follows :

(i) An XK type, resembling Malayan rural or scrub typhus.

(ii) A type embracing the cases shown in the table under the two headings X 2 and X 19 (*Poona-Ahmednagar*). There is every reason to believe that the cases analysed in these two columns are examples of the same disease, and differ in their agglutinin response because this is heterologous in nature, being an indirect reaction to a main antigen which has not yet been identified.

It is not suggested, therefore, that there is a new sub-group of which X 2 represents the main antigen.

These cases are of the type which Sir JOHN MEGAW has named " Indian tick typhus," and are related to the Rocky Mountain fever, fièvre boutonneuse group.

(iii) The X 19 (Bangalore) type, bearing a strong resemblance to endemic (flea-borne) typhus.

This classification fits into that suggested by HEATLY-SPENCER (1935) and FELIX (1935), and I venture to reproduce the first part of their table with the type " Indian tick typhus " struck out, and the three sub-groups inserted in their appropriate columns.

Sub-group.	Type X 19.	Type XK.	Type Undetermined.
Name of disease	Classical epidemic typhus. Tabardillo Endemic typhus (Brill's) of U.S.A. and Australia, Greece, Syria, Manchuria, Malaya (shop typhus) and Toulon (fièvre nautique)	Japanese river fever; (Tsutsugamushi fever of Japan, Malaya and Dutch East Indies) Malay scrub typhus Scrub typhus of East Indies	Spotted fever of Rocky Mountains. Sao Paulo endemic typhus. Fièvre boutonneuse. Febbre errutiva. Tick bite fever of South Africa. Epidemic and endemic typhus of South Africa.
Corresponding Indian groups	X 19 (Bangalore) group	XK group	{ X 2 group. { X 19 (Poona-Ahmednagar) group. (These are, clinically, so-called " Indian tick typhus.")

I agree with Sir JOHN MEGAW that the classification of this group of diseases into tick-borne, flea-borne and mite-borne non-epidemic typhus fever has many practical advantages.

As he points out, however, the severity (and presumably the clinical features) of the disease cannot be taken as an indication of difference of type. Moreover, in cases occurring in India, evidence of tick-bite (assuming that the tick can be accepted as a vector) is present in only a negligible number of patients, while in none of the military cases has there been any history or evidence showing that the patient has been bitten by mites or fleas. In fact, the existence in India of mite-borne and flea-borne typhus was not seriously considered until the use of O suspensions of the *Proteus* strains as a routine measure in the serological investigation of obscure fevers showed, by analogy with findings elsewhere, that cases of this kind were probably relatively common.

If serological reaction is to be accepted as final proof of the vector, so that, for example, a patient showing an XK reaction can, *ipso facto*, be diagnosed " mite-borne " typhus, good and well. But surely, until the connection is *proved*, this is a misleading procedure. Yet unless it is followed, it is difficult to see how a classification according to vector can be put into practice.

My suggestion, which applied only to India, was that a provisional grouping, based on serological response, should be adopted as a starting point for further observations. I still maintain that, in the absence of precise knowledge regarding local vectors, this presents the most hopeful line of approach to a difficult and complicated subject.

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Royal Army Medical College,   I am, etc.,
          *Millbank, S.W.*1.   J. S. K. BOYD.
     11*th May*, 1936.

TRANSACTIONS

Proceedings of the **29th Annual General Meeting** of the Society held at
Manson House, 26, Portland Place, London, W.1,
at 8.15 p.m., on Thursday, 18th June, 1936,
Sir ARTHUR BAGSHAWE, *C.M.G.*, M.B., D.P.H., *President*, in the Chair.

BUSINESS.

REPORT OF THE COUNCIL FOR THE YEAR ENDED 31ST MARCH, 1936.

The PRESIDENT presented the 29th Annual Report and spoke of its record
of a successful year.

Dr. C. C. Chesterman, in proposing the adoption of the Report said one
or two things in it had struck him as being very satisfactory. One was that
we had been able to extend hospitality to many other learned societies. Also,
we had enrolled 165 new members during the year.

Dr. J. W. Lindsay seconded the proposal for the adoption of the Annual
Report which was carried unanimously.

REPORT OF THE HON. TREASURER FOR THE YEAR ENDED
31ST MARCH, 1936.

Dr. Oswald Marriott, the Hon. Treasurer, in presenting his Report said the excess of income over expenditure for the year amounted to £607. He referred to the satisfactory total of £2,491 received in Fellows' subscriptions : this represented an increase of £97, and, in addition, three Fellows had paid the Composition Fee of £23 12s. 6d.

The sum realized by letting the Hall also showed an increase, and £114 had been received in donations for Manson House. During the year he had been able to reduce by £948 the total debt on Manson House, which, on 31st March, 1936, stood at £12,797. He hoped that during the present financial year even more rapid progress might be recorded.

Dr. R. Briercliffe : All who had studied the Statement of Accounts would agree that the finances of the Society were in a very flourishing condition, and he had great pleasure in proposing the adoption of the Treasurer's Report. This was seconded by **Dr. F. Murgatroyd** and carried unanimously.

ELECTION OF THE AUDIT COMMITTEE.

Dr. V. S. HODSON ; Dr. R. P. GARROW and Major J. A. CRUICKSHANK were re-elected as members of the Audit Committee.

Proceedings of an **Ordinary Meeting** of the Society held (after the Annual General Meeting) at Manson House, 26, Portland Place, London, W.1, at 8.30 p.m., on Thursday, 18th June, 1936.

Sir Arthur Bagshawe, *C.M.G.*, M.B., D.P.H., *President*, in the Chair.

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The meeting was preceded at 7.45 p.m. by a Demonstration, arranged by Dr. Hackett, of Radiographs and Specimens illustrating the subject of his paper.

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PAPER.

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BOOMERANG LEGS AND YAWS IN AUSTRALIAN ABORIGINES.*

BY

CECIL J. HACKETT, M.D., M.R.C.P., D.T.M. & H.

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The deformity of boomerang legs consists of a forward bowing of the lower limb below the knee (Fig. 1). This may be associated with some increase in the antero-posterior diameter of the tibiae. Clinical signs which are also present are the flattening of the calf and of the hollows each side of the tendo Achilles. The condition is of frequent occurrence in Central and Northern Australia, and its existence was first recorded by Stirling (1894 and 1896). Several papers on boomerang legs have appeared since that date, but most of them have done little to elucidate its aetiology. Spencer and Gillen (1899) were uncertain if it were pathological or a racial characteristic. Smith (1907) and Eylmann (1908) suggest that perhaps it is caused by rickets. Breinl and Priestley (1915) report a case and postmortem findings. They exclude syphilis and tuberculosis

as causal factors, but wrongly describe the specimen, which is in the museum of the School of Tropical Medicine at Sydney, as consisting almost entirely of compact bone. This error has been quoted by CASTELLANI and CHALMERS (1919), who also refer to the paper by CHRISTOPHERSON (1918) describing a case from the Sudan which was probably not one of boomerang legs. They say that a condition clinically similar to boomerang legs is undoubtedly a late manifestation of yaws ; and they consider that neither rickets nor osteomalacia plays any part in the production of the deformity. BASEDOW (1925) thinks that the tibialis posticus may be a factor but mentions no associated conditions. HERMANS (1928 and 1931) suggests that boomerang legs may be identical with the " sabre tibiae " which result from yaws. Various authors have emphasized that the deformity is found in individuals who appear to be quite healthy.

In 1933 a native child was seen who had several diseased bones and joints, extensive skin ulceration and boomerang legs. It was suggested that perhaps the leg deformity was associated with the other conditions and that they were all due to yaws. The work in 1934, in the first instance, was planned to test the validity of this association. The papers by HERMANS (1928 and 1931) and CASTELLANI and CHALMERS (1919) were not seen until work in the field had been commenced.

THE AETIOLOGY OF BOOMERANG LEGS.

In 1934 clinical, radiographical and serological studies were begun at Alice Springs. Here observations were made on fifty aborigines of a population of about 400. It was found that certain scars were often present about the mouth and in the joint skin flexures. (Fig. 2.) These scars were characterized more by a loss of elasticity of the skin than by previous extensive destruction. There was usually no alteration in the pigmentation of the scars in full-blood natives. who attributed them to an earlier attack of a disease that they called *irkintja*. SPENCER and GILLEN (1899) record a legend of the existence of this disease in the mythologically distant times and the belief that certain magical processes can cause the disease to break out among any whom one wishes to harm, EYLMANN (1908) also writes of this condition. It is said that it only attacks an individual once, and appears in the moist areas of the body. EYLMANN thought that it was not syphilis but confuses it with a number of tropical skin lesions. BASEDOW (1932) rather indefinitely indicates that this disease and yaws are the same but offers no evidence in support, except that the natives knew both the secondary eruption of yaws and gangosa by the same name, *irkintja*. Many subjects at Alice Springs also gave a history of having suffered from pains in the shins before the onset of boomerang legs. The clinical course of the development of the deformity may be epitomized as follows : During infancy or early childhood the subject contracts the disease, *irkintja*, after this clears up there is a quiescent period of a few years ; then pains in the shins are experienced and the legs become bent.

This rarely occurs after puberty and it is probable that no increase in the

deformity takes place after the epiphyses are fused. The kinship of some of the cases is probably accounted for by the presence of *irkintja* as an endemic disease, rather than that the deformity is hereditary. In many instances the Wassermann reaction was positive. On analysing the data the only significant association was found in the presence of scars and the history of a previous attack of *irkintja*. There were, however, indications that other associations were also
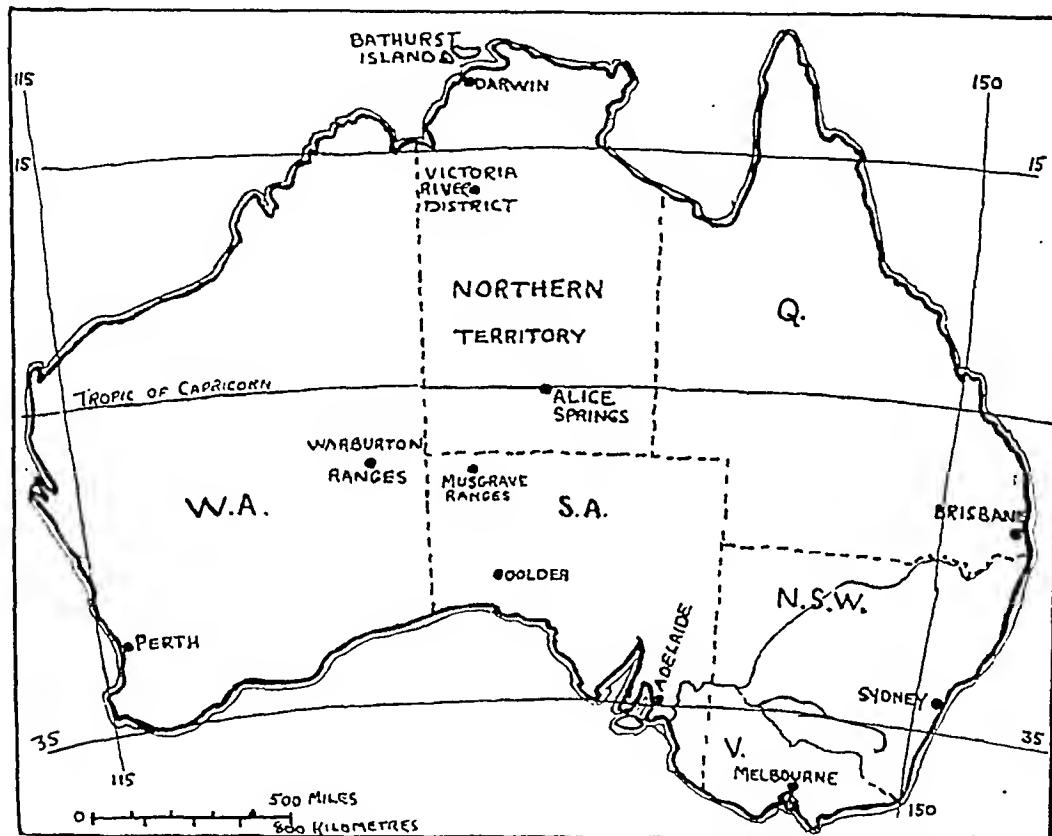
MAP OF AUSTRALIA SHOWING AREAS IN WHICH INVESTIGATIONS WERE UNDERTAKEN.

present. The association of scars and boomerang legs was upheld by observations in the Victoria River District and at Bathurst Island, where of 322 subjects, 49 per cent. had boomerang legs and scars, 9 per cent. had boomerang legs only, 5 per cent. had scars only and 37 per cent. had neither condition. The association of positive Wassermann reactions in the sera and boomerang legs was supported by observations at Darwin and in the Warburton Ranges, Western Australia. Among the inmates of the Darwin Half-Caste Institution, five with neither boomerang legs nor scars were found to give negative Wassermann reactions in their sera, while five with boomerang legs and scars gave positive results. Of thirty-six males in the Warburton Ranges, over the age of 15 years, seventeen

(44 per cent.) gave positive Wassermann reactions. Of seventeen cases of boomerang legs fifteen (88 per cent.) had positive reactions and one of the remaining two cases had a chronic arthritis of the knee.*

The scars, which have been referred to, were recognised by medical men in the Northern Territory as resulting from yaws. As syphilis is unknown among the aborigines in this area, it may be concluded that the positive Wassermann reactions in the sera of these cases are due to infection with yaws ; and that yaws is the cause of boomerang legs.

GEOGRAPHICAL DISTRIBUTION.

It is found that boomerang legs and yaws occur throughout the Territory. BREINL (1912), HOLMES (1912) and BREINL and HOLMES (1915) report that yaws was frequently seen in the northern coastal districts and BASEDOW (1932) reports its presence in Northern and Central Australia. BASEDOW also encountered it in north Western Australia, and it occurs in Northern Queensland. In Australian museums are specimens of boomerang tibiae from localities which cover the eastern half of the continent. In a recent critical survey (HACKETT, 1936) of references to syphilis and " venereal disease " among aborigines from early medical and lay writers, dealing especially with the south-eastern quarter of Australia, it was found that (if occasional cases of gonorrhoea and granuloma pudendi were excluded) these descriptions are probably misdiagnoses of yaws. In most tropical countries, where yaws is endemic, sabre tibiae are also reported. Thus the co-equal distribution of boomerang legs and yaws in the Northern Territory at present, and more extensively in the past, supports the conclusion that this deformity is none other than the sabre tibiae of yaws.

PATHOGENESIS.

The fifty cases that were studied at Alice Springs were arranged, according to the degree of the deformity, into three groups. From the radiographs of the group with the most marked bowing, it was possible to follow the course of the development of boomerang legs. This was supported by the findings in the other two groups.†

It was found that the earliest stage was identical with lesions described by MAUL (1918). These consist of multiple small areas of rarefaction in the cortex and medulla. In the case in which these were present, the tibiae were already bent and the zones of condensation round the rarefied areas were taken to

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*Through the kindness of the Director of the Adelaide Hospital Laboratory, the Wassermann reactions were carried out by Mr. F. S. TEE.

†I am indebted to Messrs. W. WATSON & SONS (Melbourne) for the loan of a Caldwell portable X-ray outfit, to KODAK (Australasia) for the provision of a supply of films, to the Post Office authorities for the electrical supply and accommodation at Alice Springs, and to Mr. C. H. MARSHALL, of the Adelaide Hospital, for his excellent services as radiographer.

FIG. 1.

Marked boomerang legs in a male aborigine aged about 28 years.

FIG. 2.

Scars about the mouth in a half-caste boy.

Fig. 3.—Radiograph of case illustrated
in Fig. 1.

Fig. 4.—Multiple necrotic foci in
Specimen 1031. Pathological Museum,
Adelaide University.

indicate that the process was resolving. It is suggested that it is during this stage that the weakened bone is deformed by body weight and muscular action. As resolution takes place the cortex becomes thickened and the marrow cavity is encroached upon, so that the cortical shadows may occupy the greater part of the antero-posterior diameter of the bone. With the further passage of time the thickened cortex is converted into cancellous tissue from the medullary surface and in time may be reduced to normal proportions (Fig. 3). This may account for the statements of some of the earlier writers who spoke of a bone which was normal except that it was bowed. In some of the more severe cases the nutrient canals were notably enlarged. In some tibiae there were areas of sclerosis, transverse linear shadows in the medulla of the shaft and lines of arrested growth. The last two changes are non-specific and may be taken as indications of general disorder in the health of the subject as a whole rather than peculiar to any bone disease. In those who had not reached puberty the epiphyseal regions were normal. The above interpretation of the radiographical findings is based on the examination of museum specimens. The appearance presented by any case of boomerang legs would depend on the severity of the initial lesions and the time that had elapsed since the onset. The absence of marked periosteal deposits would differentiate the condition present from syphilis.

OTHER BONE LESIONS.

In the radiographs of the Alice Springs series three types of lesion were found which did not fit into the development of boomerang legs. These three types may be described as : (1) multiple necrotic foci, 5 to 10 mm. in diameter in long bones associated with expansion of the bone and some relatively slight periosteal reaction (Fig. 4), but there may be no reaction in flat bones. (2) periosteal nodes with or without necrosis, (3) generalised periosteal deposits. Similar lesions are to be found in aboriginal bones in many museums both in Australia and Great Britain, and at present are usually regarded as syphilitic.* However, taking into account the absence of syphilis among the autochthones of Australia and the presence of these lesions in the Alice Springs series, it is suggested, until further work has proved otherwise, that they are probably the result of yaws. Sir ROBERT MUIR kindly examined several of these specimens and said that while the changes exhibited by the third type did not differ from those due to syphilis, he had not seen syphilitic lesions resembling those of the first type. In a tibia in the Royal College of Surgeons, London (3974·1) in which there are generalised periosteal deposits, there are also necrotic foci, which indicates that these two types of lesion may have a common causal factor. The periosteal deposits in some cases may result from secondary infection of previously diseased bone, through open lesions. Trauma and superinfection may play a part in determining the situation and type of lesion that is produced.

*Unless otherwise noted these specimens are in the Wellcome Medical Museum, London.

In the skulls of aborigines in museums several kinds of lesion are met with. In a specimen from Victoria the lesions of gangosa are present. In a skull from Adelaide there are multiple small areas of erosion. A skull from the lower River Murray district has several areas, 1 to 2 cm. in diameter, of necrosis in the calvarium. In some of these areas central sequestra have formed while in others these have separated and cavities have appeared. In two Australian skeletons in the Royal College of Surgeons (20·7342 and 20·6508), the skulls show similar massive necrotic foci while other bones exhibit lesions comparable with those described above. This, together with the considerations already referred to, leads to the suggestion that these changes may also be due to yaws.

It is regretted that no histological material is available, but most of the descriptions of yaws bone lesions in the literature can be fitted into the above types. In conclusion, attention might be drawn to the work of SCHOEBL (1928) where he suggests that the bone lesions of yaws are probably the result of a process analogous to that of keratoderma plantare, which he regards as a late framboeside, the result of only partial immunity. TURNER, SAUNDERS and JOHNSTON (1934) report the failure to find spirochaetes in yaws bone lesions.

SUMMARY.

(1) The yaws origin of boomerang leg in Australia is demonstrated.

(2) The widely spread distribution of yaws and boomerang legs in Australia is recorded.

(3) The development of boomerang legs is outlined from radiographical and morbid anatomical findings.

(4) Certain other bone lesions of Australian aborigines are described which, it is suggested, are the results of yaws.

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DISCUSSION.

Prof. H. A. Harris (of Cambridge) : Dr. HACKETT has presented a series of clinical events which is disturbing. We have heard much of transitory rachitic lesions in bone in childhood, but here we have a condition which is new to all of us inasmuch as the condition is traced both as regards the skin lesion, the bony lesion, the deformity and the radiographic appearance from infancy to adult life. The boomerang tibia is a deformity which does not fit in with any of the deformities seen in orthopaedic clinics in this country. The bending of the tibia is so marked and yet so gradual and regular ; the antero-posterior curve is strictly limited to the sagittal plane. The curve is clean and free from any irregularity or epiphyseal enlargement. The condition in the sabre tibia is distinct from any condition known in this country.

As to the general features of the Australian skeleton, one is struck in the first place by the shape of the skull, which from the posterior aspect, appears as a pentagon—like a haystack seen end-on. Another characteristic is the poor development of buttocks. The small buttocks are associated with a small femoral head, long thin femoral shaft, absence of heavy muscle markings— especially for the gluteus maximus. Any series of European long bones, if examined with care, can be graded so as to yield three main types. In the first group are the bones with a stout shaft and large epiphyses. In the second group are finely made bones with a slender shaft and small epiphyses, whilst the third group presents a shaft which is slender over the whole extent but broadens out suddenly into enlarged epiphyses. This last type presents marked tubulation of the shaft with the maximum of bony absorption at the metaphyses during the

growth period. The long bones of the Australian definitely belong to the second group, with long slender shafts, small epiphyses and extreme tubulation so that the bone as a whole is reduced to the minimum bulk.

If you consider the native in the sitting position with elbow on knee, it is possible that any great degree of muscular wasting, involving the extensor before the flexor muscles, might give rise to an antero-posterior bowing of the femur or tibia as a result of the unopposed action of the hamstrings and the flexors of the calf. Similarly, extreme wasting in the forearm might give rise to the curvature of the radius and ulna on account of the unopposed action of the flexors. The production of such a static deformity by muscle wasting still fails to explain the side to side flattening and bony absorption in the tibia.

What condition might give rise to the extreme economy in the handling of the inorganic elements in the bone, possible disturbances in antagonistic groups of muscles, and consistent uniformity in the type of bony deformity ? We may be dealing with a phosphorus deficiency, for muscle contains far more phosphorus than calcium. The phosphorus shortage may be a definite factor in the production of the bony deformity. The presence of an infection such as yaws may aggravate the effect of the phosphorus shortage.

Dr. HACKETT has demonstrated a condition which calls for a detailed study of bone pathology with special reference to yaws and the possible role of phosphorus or other deficiency.

Dr. Mather Cordiner : Dr. HACKETT is to be congratulated on his very excellent series of radiograms, a series which I think, must be unique. Such X-ray appearances as we have been shown this evening are not met with in this country, but would, almost certainly, be regarded as syphilitic. There is one point, however, which is strongly against the lesion being syphilitic. In the congenital syphilitic lesion the chief characteristic is a periosteal reaction with very little bone atrophy. As a result deformities do not take place and one does not encounter a bending of the bone with the syphilitic lesion. In the older person the lesion shown is not unlike an osteitis deformans but in this condition there is always a very marked increase in the bone trabeculation, while in none of the radiograms of boomerang leg shown is this trabeculation apparent.

Professor Warrington Yorke : It is a mystery to me that these people do not get syphilis seeing that they suffer from gonorrhoea , unless *irkintja* immunizes against syphilis. I am sorry that Professor BLACKLOCK is not here, as he holds very definite views on the relationship of syphilis to yaws. It is curious that the same infection in different parts of the world seems to produce, at times, different lesions. If *irkintja* is yaws, why does it not exhibit the classical manifestations of this disease which one sees in other parts of the world ? I have been struck with a similar fact in respect of filariasis, which, in some parts of the world, produces predominantly chyluria, in other places elephantiasis of

the legs, and in still other parts elephantiasis of the arms. If all these conditions are due to filaria, one can only assume that the habits of life of the people, and possibly their diet, has something to do with the kind and position of the lesion produced.

I do not know that it is any real argument to say that boomerang leg is yaws because you have not found in this country similar bone lesions in syphilitics. We might as well argue that *irkintja* cannot be yaws because no one has as yet seen in it the typical cutaneous lesions of yaws. The whole subject is of great interest and urgently requires further investigation.

Dr. H. S. Stannus : I am glad Dr. HACKETT has drawn attention to the fact that so much of our knowledge concerning yaws lacks exactitude ; in regard to "boomerang leg," he has made a valuable contribution towards defining the evolution of that affection.

Among other points to which he has drawn attention is the question of the geographical distribution of yaws in Australia. Basing his conclusions upon the fact that "boomerang" tibiae have been recovered from all parts of Australia, Dr. HACKETT says that yaws occurs and has occurred in native groups living in non-tropical climates and in areas far removed from the defined geographical tropics ; and he suggests that conditions other than atmospheric temperature and humidity play a part in the etiology of the disease, the special factor being scantiness of clothing. It is worthy of note however that the area of Australia demarcated in his map, that in which yaws is found, will be seen to correspond very closely with the area enclosed by the isotherms which delimit the tropical belts of other countries, and I suggest that this is a fairer way of defining " the tropics " than by making use of the tropics of Cancer and Capricorn. Alice Springs is almost on the tropic of Capricorn, Musgrave Ranges are well within the geographical tropics and Warburton Ranges only 26° S. That absence of clothing is perhaps the most important etiological factor there can be little doubt ; it is a view I expressed elsewhere last year when I suggested that it was the conditions associated with a tropical climate rather than the climate *per se* which governed the distribution of yaws : primitive peoples, living under primitive conditions in regard to clothing, housing, feeding, cleanliness and protection from traumata. Thus are explained the wide diffusion of yaws among bush natives, its absence among those who dwell in towns, the comparative freedom of the white man, childhood infections, etc. Where similar conditions are met with outside the tropics there I think yaws might spread if introduced, as is well exemplified by the outbreak of yaws in a Johannesburg mine (C. J. SCOTT, 1933) where, though the altitude of Johannesburg is 6,000 ft., work was carried on 1,000 to 2,000 ft. below sea level at a temperature of 88° to 92° F. dry bulb thermometer and 87° to 91° F. wet bulb. The miners worked, stripped to the waist, but wearing boots, and it is of interest to observe that primary

lesions occurred on every part of the body and limbs except the foot and ankle, also that white miners were infected as well as native.

In regard to "bomerang leg" itself the point which strikes one most forcibly is the extremely high incidence. At Alice Springs alone, Dr. HACKETT collected fifty cases where, let it be remembered, the population all told only numbered a few hundred, this sparsity of population being a point which perhaps Dr. HACKETT has not emphasized. In the Northern Territory, of 322 persons no less than 58 per cent. exhibited the condition. In no other country, as far as I know, in which yaws is endemic, is there such a high incidence of this or of the similar bone deformity known as sabre tibia. One is I think forced to the conclusion that some other factor must be involved, as Professor HARRIS has suggested. This high incidence of a particular lesion reminds one of the high incidence of goundou among the yaws-infected primitive natives of the forest zone of the Ivory Coast as reported by BOTREAU-ROUSSEL. There surely must be a special factor at work. That concurrent infections, deficiency states and endocrine disturbances play a part in determining the course of syphilis is well known, and I think the same may be true of yaws. The subjects of Dr. HACKETT's observations are, I gather from him, a people with a very precarious food supply and unbalanced dietary. It would seem quite possible that some deficiency is the factor determining the local incidence of boomerang legs.

Just how common bony lesions are in yaws we do not know; serial X-ray examinations of a large number of cases of yaws throughout the course of the disease are required : they were not uncommon in association with goundou in BOTREAU-ROUSSEL's cases.

I gather that Dr. HACKETT regards the pathological changes in boomerang tibia as differing from the picture presented in syphilis; but I am not quite clear whether he thinks the presence or absence of periostitis a point of differentiation. Osteo-periostitis is common in yaws—if periostitis is rare or absent in boomerang leg it would perhaps be another point in favour of there being a special factor involved in that condition. Bone lesions due to yaws, in an otherwise normal individual, might differ from those occurring in one suffering from a condition of sub-scurvy. Trauma may certainly play some part but the evidence for believing that superinfection may have an effect in producing bone lesions is I think unconvincing. I am not quite clear on what grounds it is suggested that bone lesions may be a manifestation of partial immunity. I could understand their being, like gummatous lesions, possibly an expression of the allergic state—a very different thing.

Many of the bony exhibits show evidence of marked necrotic lesions. It would be interesting to know whether they were associated with ulcerative lesions of the skin and secondary infections.

It would be interesting also if Dr. HACKETT would add some observations concerning the lesions on the skulls shown. As far as I can remember only once have I seen it suggested that yaws causes lesions of the vault of the skull.

Dr. G. Carmichael Low said that from what Dr. HACKETT had told them, it was clear that boomerang legs had some connection with yaws. The only point of criticism that might be put forward was that Dr. HACKETT had not seen ordinary yaws amongst the children and young adults of the aboriginal natives. That might be explained of course by the difficulty of seeing sufficient cases in such a sparsely distributed population.

He (Dr. LOW) agreed with Dr. STANNUS that there must be some other factor present to make the bones so soft as to allow of this special bending seen in boomerang leg. In countries where yaws was very prevalent, one did see from time to time examples of sabre tibia, but not in great numbers, and the same held good for some other conditions now generally attributed to yaws. For example, if goundou was a sequel of yaws, why was it limited almost entirely to natives in some areas of the West Coast of Africa, and practically not seen elsewhere, and why was gangosa specially found in Guam ? Again juxta articular nodes were more common on the West Coast of Africa than elsewhere. All these examples pointed to some other factors, in addition to the treponema of yaws, influencing the development of these special pathological conditions.

The question of syphilis and yaws was a very interesting one ; they had a close similarity, but in his (Dr. Low's) opinion, they could be differentiated clinically with ease.

Other anomalies which required clearing up, as regards these two diseases, were the absence of syphilis and the great prevalence of yaws amongst the Fijians ; and now, according to Dr. HACKETT, the absence of syphilis amongst the aboriginal natives of Australia and yet the presence of yaws.

As regards the bone shown with the fenestrated holes in it, he again agreed with Dr. STANNUS that there must have been open ulceration of the skin present over the lesion, and that cocci had played a part in its production.

He hoped that Dr. HACKETT would be able to continue his work on yaws, and that he might have the opportunity later of seeing the disease in other parts of·the tropics where it was specially prevalent.

Dr. Clement C. Chesterman : I, too, have been very much interested in this discussion on yaws, because in the Belgian Congo the disease is very widespread, and during the last three years we have treated 22,000 cases of yaws at the Baptist Mission Hospital of Yakusu and its village dispensaries.

Dr. HACKETT has staked out a good claim for the presence of yaws in the Australian continent. There is no doubt that these bony lesions are very similar to those we have in the Congo. There the natives call them " machete legs " ; the machete being the equivalent of the sabre. But I have not seen such marked bowing and thinning as Dr. HACKETT has shown us. There seems to be more sclerosis, due probably to the better food supply in the rain forest than in the Central Australian districts. Also I am sceptical about the specimen from the Royal College of Surgeons Museum. I have spent many hours hacking away

the involucra from old cases of osteomyelitis, and I am sure that condition occurs frequently among natives. The natives of the Congo have good resistance to septic infections, but they are not so immune to it as the Australian natives seem to be. I have not observed scars on the skin, except after the superficial tertiary ulcers. The primary lesion, unless infected with the organisms of sloughing phagedena, does not leave much scarring of the skin, neither does the secondary lesion. Most of the osteo-sclerosis in this condition is on the anterior or convex surface, and is frequently seen in the forearm bones, the clavicle and tibia, and so I wondered whether a mechanical explanation would not serve, the convexity of a slightly curved bone being under tension both when bearing weight or through muscular action, while the concavity would be under compression.

I ask whether Dr. HACKETT, or any other Fellow of the Society, has any idea why some people do not get this bony lesion ; why should tertiary yaws develop in some cases and not in others ? I have never found this or any manifestation of tertiary yaws in patients who have had some chronic secondary lesion, such as crab yaws or other persistent secondaries which refuse to heal. This suggests, and native opinion strongly confirms it, that a good cutaneous reaction is the best safeguard against tertiary lesions.

I can support Dr. HACKETT's experience that syphilis is not always prevalent where other venereal diseases may be found, and we have also had cases in the Congo in which typical syphilis has been contracted after typical yaws.

The President, Sir Arthur Bagshawe : One thing which occurred to me, as it probably has occurred to others, is that with such an amount of bowing there may be a liability to fracture. If there is a dietetic factor, one would think it would leave some brittleness of bone.

Dr. Hackett (in reply) : The yaws origin of the lesions in the skulls in the demonstration is only a suggestion based upon the absence of syphilis and pyogenic infections and the frequency of yaws among the aborigines. Further evidence is needed for confirmation of this probability.

Some of the periosteal deposits round open lesions may be due to secondary infection in de-vitalised tissue. And the same remark applies to the lesions in the skulls, which are probably associated with sinuses though I have not any living cases. Of the massive necrotic condition there are six specimens in the Royal College of Surgeons Museum.

I do not think fracture often occurs in these bent bones. If one leg is more bowed than its fellow, a history is frequently given of some trauma in early life, such as a fall or some knock ; I think such traumata must play an important part in the determination of the localisation of the lesion.

As to cross-immunity between syphilis and yaws : from the few cases I have seen reported of yaws, which later developed syphilis, the first disease was

of mild character. One such case was recently reported from Kenya. In that, the attack of yaws was so mild that the patient had taken but little notice of it, when however, he developed a syphilitic chancre he sought medical advice. In SCHOEBL'S work on cross-infection between these two diseases in animals, it was found that to get immunity to syphilis it was necessary to keep the yaws lesion going for at least 4 months : after that period the chances of infection with syphilis were greatly diminished. Earlier, however, there was a fair chance of such infection.

With regard to the persistence of secondary lesions hindering the production of tertiary ones, SCHOEBL found with his monkeys that if in the early inoculations a good metastatic eruption developed the animals would not develop late lesions.

The change which occurs on the convex surfaces of the boomerang tibiae opens up the question of mechanics and the operation of bone trajectories.

The scars, which Dr. CHESTERMAN has not seen frequently in the Belgian Congo, are found, in the regions of Central Australia where the climate is drier, in the skin creases in infancy round the neck, the gluteal cleft, and the axillae and the ante-cubital fossae. At Bathurst Island, where there is a good deal of hookworm disease, the incidence of these scars round the mouth was increased. There the habit of earth-eating was frequent. I do not know whether the trauma of the earth passing over the lips would be responsible for the localisation of the lesions, or whether it is due to fingers carrying infection from elsewhere on the body.

With regard to the influence of secondary infections in the production of these lesions, septic infections of the soft tissues are not frequent and they heal very readily. Deep depressions are often found over the left scapula, the results of fighting, but they are probably due to thorough undercutting and sloughing, rather than to infection. I have seen only one serious infective process in a native, a facial carbuncle which developed in 2 days ; in another 2 or 3 days it had resolved.

On the question of the distribution of yaws being due to some factor other thàn a climatic one, I have wondered whether the common factor is not skin exposure. In the tropics there is the factor of warmth ; among the Australians there is ethnological backwardness. The winter nights in Central Australia are cold, the temperature often falling below freezing point.

The distribution and character of the lesions of yaws seen in Central Australian natives fall into line fairly well with those reported by RAMSAY (1925) in Assam. In the dry hill country the lesion is a dry scab localised to the moist areas in the flexure regions of the body ; but in the lower-lying moist climate, there is a more general distribution. When the hill people come down to the moist country, they are found to develop the typical yaws.

I agree with Professor WARRINGTON YORKE concerning the inability of comparison of these lesions in Australian aborigines with syphilis in Europeans. There is little opportunity of studying Australian aborigines, under full European

conditions.   But much can be done by a comparison of negro syphilis in the northern part of the United States, and the bone lesions of yaws in Africa.

Professor HARRIS and Dr. STANNUS's suggestions of an extra factor and deficiency are sound and it would be interesting to get early active cases, selected by radiography, and to study their calcium and phosphorus balances and their blood phosphatase values.   If these lesions are not due to syphilis but are due to framboesia, that is some indication that these diseases are not identical.

It is reported by TOPINARD (1890), that 16 per cent. of the tibiae taken from graves in Paris, of the 4th to the 10th century, are both flattened and bent in an antero-posterior direction.   It would be interesting, if rickets could be excluded, to see how these deformities compare with those in boomerang legs. EATON (1916) speaks of syphlitic lesions in Peruvian bones from burials before the European discovery of the country.   The differential diagnosis was not very thorough ; osteitis deformans was excluded in one instance on account of the youth of the subject and it was concluded that the lesions present were the result of syphilis.   Yaws was not considered.   The changes present in this case are not unlike those present in some of the Australian bones, and do not resemble the usual syphilitic lesions.

I have had little opportunity this evening to acknowledge my indebtedness to the many, both in the field and in the cities of Australia and in Great Britain and elsewhere, who have so freely and willingly given me any assistance needed, and I take this opportunity of expressing my deep gratitude to them.

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COMMUNICATIONS.

YELLOW FEVER IN THE GAMBIA.

II.—THE 1934 OUTBREAK.

BY

G. M. FINDLAY

AND

T. H. DAVEY.*

*Wellcome Bureau of Scientific Research, London ; and Sir Alfred Jones Research Laboratory,
Liverpool School of Tropical Medicine, Freetown, Sierra Leone.*

In a previous communication (FINDLAY and DAVEY, 1936) a brief historical account was given of the various outbreaks either of yellow fever itself, or of diseases closely resembling yellow fever, that had occurred in the Gambia since its discovery by Europeans 480 years ago.

In the present communication observations are recorded on the epidemic that occurred in Bathurst during the last 3 months of 1934.

A few preliminary details in regard to the town may not be without interest.

Bathurst, the capital and only town of any size in the Gambia, was founded in 1816 on the island of Banjola, thereafter renamed the island of St. Mary. The site of the town was apparently selected on strategic rather than on sanitary grounds for the island, 13° 27′ N. latitude, 16° 34′ W. longitude, is in reality little more than a sand-bank approximately $3\frac{1}{2}$ miles long and $1\frac{1}{4}$ miles broad. The island lies 18 miles from the mouth of the River Gambia which is here some $2\frac{1}{2}$ miles wide ; it is bounded on the north by the main stream of the river and is separated from the mainland to the west by a narrow channel, Oyster Creek. The island has an area of approximately 2,500 acres, but a large part is swampy and during the rainy season water-logged, since the highest point on the island is only about 6 feet above sea-level, while much of the island is from 3 to 4 feet below sea-level. Even in the middle of the dry season subsoil water can therefore be reached by digging down a few feet. As a result

of the physical configuration (Fig. 1) efficient drainage of the island is at present almost impossible, the difficulties being increased by the fact that, apart from an occasional shower in January, the rains are confined to the months of June, July, August and September. Though the rainy season begins and ends with tornadoes, from a half to a third of the year's rains occur in August.

Considerable variation is found in the annual rainfall ; in 1905 for instance, 66·07 inches fell, but in 1913, only 23·68 inches ; the average is about 45 inches.

No correlation exists between the incidence of yellow fever and the annual rainfall either for the year in which yellow fever occurred or for the preceding year. The possibility that certain general conditions were indirectly responsible for the epidemic is, however, suggested by the fact that during the last 3 months of 1934, outbreaks of yellow fever also occurred in and around the town of Kano, Northern Nigeria, in Hill Station, Sierra Leone, and in an isolated prospecting camp in the Gold Coast.

Wide diurnal variations in temperature, sometimes amounting to 30° F., occur during the dry season in the Gambia. The harmattan blows intermittently from the Sahara from December to April.

The population of Bathurst, as given in the 1931 census, was 14,370. In 1840, 24 years after the foundation of the town, the population was only 2,825 Africans and 36 Whites. The increase has been due not to an excess of births over deaths, for only in 1909, for the first time on record, was the number of births slightly in excess of the number of deaths, but rather to immigration from the adjacent countryside.

Although Bathurst was carefully laid out with broad straight streets, the increased population has resulted in very considerable overcrowding (Fig. 2). Complete segregation of Europeans, advocated on numerous occasions, is under present conditions clearly impossible and in addition, many of the houses, more than a hundred years old, are unsuitable for Europeans. Even in 1927, two fresh European houses were erected in the heart of the African quarter.

The principal agricultural export, on which the prosperity of Bathurst and the Protectorate depends, is the ground nut (*Arachis hypogaea*).

The Yellow Fever Outbreak in 1934.

For the first 9 months of 1934, as judged by the monthly returns of deaths (Table I), there was no abnormal degree of sickness among the African population of Bathurst. On 6th August, however, an African female, F.R., aged 32, Aku tribe, was admitted to hospital with fever, severe headache, pains across the small of the back and aching in the limbs. The urine contained albumin : no malarial parasites were found in the blood and nothing abnormal in the stools. The pulse was not characteristically slow. The temperature returned to normal on 17th August without a definite diagnosis having been reached. The patient's blood, when tested 5 months later, was found to protect in a dilution of 1 in 128. At some time, therefore, she had certainly suffered
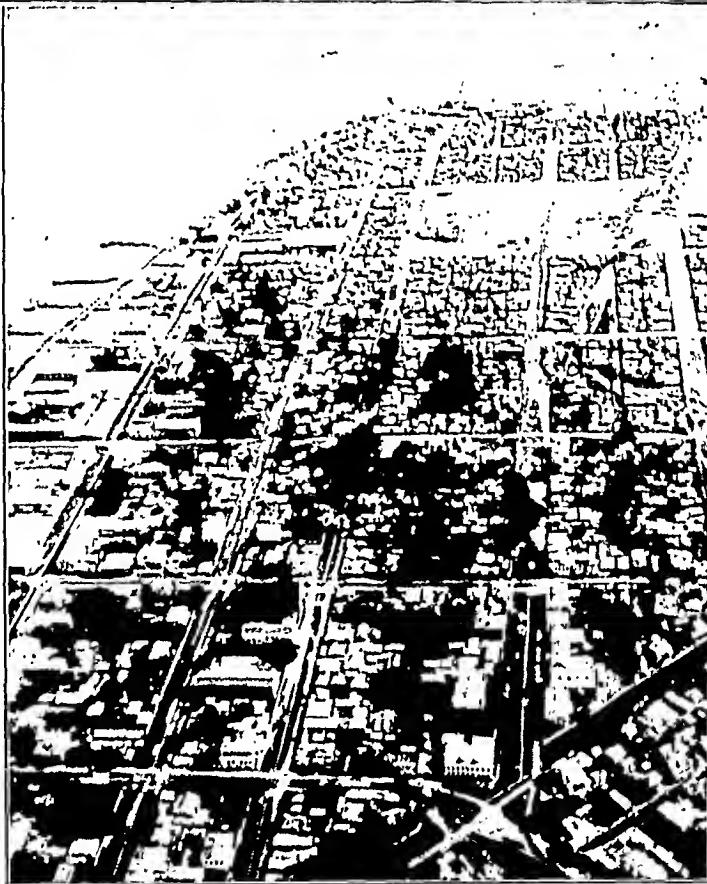
FIG. 1.—THE ISLAND OF ST. MARY, GAMBIA.
Note the swamps surrounding Bathurst.
FIG. 2.—BATHURST, GAMBIA.

from yellow fever though whether this was in August, 1934, cannot, of course, be determined.

In September the number of deaths showed a slight, though hardly a significant, increase over the figures for 1933 and 1932—thirty-six as compared with thirty and twenty-four respectively. During October, November and December, however, the number of deaths both of adults and of infants under five showed a definite increase, more marked among adults than among infants. (Table 1.)

TABLE I.

DEATHS IN BATHURST DURING 1932, 1933 AND 1934.

	Under 5 Years.			Over 5 Years.		
	1932	1933	1934	1932	1933	1934
January	4	—	3	22	26	22
February	—	1	—	21	12	17
March	—	—	1	13	23	24
April	1	—	—	20	14	20
May	2	2	—	27	20	29
June	3	1	3	14	20	25
July	4	1	2	14	17	16
August	11	6	4	19	24	17
September	5	4	8	19	26	28
October	8	3	10	19	24	32
November	3	2	5	24	22	29
December	1	1	3	19	22	46
1st quarter of year	4	1	4	56	61	63
2nd ,, ,,	6	3	3	61	54	74
3rd ,, ,,	20	11	14	52	67	61
4th ,, ,,	12	6	18	62	68	107
Total for year	42	21	39	231	250	305

Unfortunately, many Africans dying in Bathurst are unattended medically, so that a majority of the diagnoses are approximate only. Two deaths, however, were certified as due to uraemia, one to acute nephritis and one to acute malaria.

Case 1. The first patient (Table II) suspected as having died from yellow fever was taken ill on the morning of 3rd October and died 3 days later. She dwelt in an old house in Buckle Street, unscreened as are most of the European dwellings in the town. Deaths from yellow fever had occurred in the near neighbourhood in 1928 and in 1911. In fact the whole distribution of the cases in 1934 is strikingly similar to that of the 1911 outbreak.

TABLE II.

CASES OF YELLOW FEVER NOTIFIED IN BATHURST.

(OCTOBER, 1934—JANUARY, 1935.)

Case No.	Sex.	Nationality.	Date of Onset.	Date of Death.	Duration of Illness in Days.
1	F	French	3.10.34	6.10.34	3
2	M	British	31.10.34	5.11.34	5
3	M	,,	8.11.34	12.11.34	4
4	M	,,	14.12.34	19.12.34	5
5	M	African	22.12.34	25.12.34	3
6 ?	F	,,	1.1.35	Recovered : protection test negative	

Case 2. The second case, also fatal, did not occur till 4 weeks later. The patient died on the 5th day of illness. The residence of this patient was on the Marina quite close to the bungalow of Case 4. The third patient resided within 100 yards of Case 1. He became sick 8 days after the onset of illness in Case 2, and died on the 4th day of illness. An interval of 32 days elapsed between the death of Case 3 and the appearance of the fourth case. The fourth patient died on the 5th day of illness.

The clinical symptoms and pathological changes in these four patients leave little doubt that they died from yellow fever. The same certainly does not apply to Cases 5 and 6. The history of these patients is briefly as follows :—

Case 5. J.G., African male, aged 29, was admitted to the Victoria Hospital, Bathurst, complaining of enteritis. Repeated examinations of his stools did not reveal the cause of the intestinal symptoms. On 22nd December, he was given carbon tetrachloride by mouth ; the same evening his temperature rose to 101·4° F., but returned to normal on the following day though he complained of general aching and intermittent headache. On 24th December, his temperature again rose and he began to vomit coffee ground material at intervals. His urine contained albumin and granular casts, and during the last 40 hours of his life only 8 ozs. of urine were passed. He died at 11.55 p.m. on 25th December.

Postmortem.—The conjunctivae were icteric, the subcutaneous fat orange coloured : the liver was pale, with haemorrhagic patches under the capsule : the spleen was enlarged and congested : the kidneys pale. The histological changes were such as might have occurred in acute carbon tetrachloride poisoning. Definite evidence that death was due to yellow fever is thus lacking.

Case 6. V.M., African female, aged 6, was admitted to the Victoria Hospital early in December, 1934, with ulcers on the legs and arms. On 1st January, 1935, she complained of abdominal pain and vomited clear fluid in large quantities : the temperature was 101·4° F. The fever reached 104·4° F. on 4th January, and was normal 6 days later. On the 3rd day coffee ground matter was vomited and on the following day the conjunctivae were icteric. Albumin and casts were present from the 2nd to the 9th day of illness. Blood was removed 18 days after the onset of illness and tested for immune bodies to yellow fever ; none were present. It is therefore probable that this illness was not yellow fever.

Although only the above six cases were notified as having actually, or probably, suffered from yellow fever there is evidence which strongly suggests that at least six other Europeans suffered from yellow fever during the months of October and November. With one exception all were resident on the Marina, in close proximity to the quarters occupied by Cases 2 and 4. The symptoms in these six cases were attributed either to influenza or to malignant tertian malaria though parasites were not found. The clinical picture in all cases was very similar, fever, headache, aching in the back and legs and vomiting, though coffee ground material was absent. In three out of the four cases examined albumin was present in the urine. The temperature fell to normal usually about the 6th or 7th day of illness and convalescence was uneventful. In one case the skin was said to have had a slight icteric tint and in three the pulse during early convalescence was slow. Clinically, therefore, the cases bore considerable resemblance to mild cases of yellow fever.

Additional, though not conclusive, evidence that yellow fever was the disease from which these six Europeans suffered was obtained from an examination of their bloods for the presence of immune bodies to yellow fever.

Blood from five of these patients, examined early in January, showed immune body titres, by the mouse protection test, of 1 in 256 in one case, 1 in 128 in three and 1 in 64 in one. In November, in common with 131 other inhabitants of Bathurst, these five persons received an injection of mouse brain infected with neurotropic yellow fever virus, the virus having been "attenuated " for 4 days at 22° C. No reactions followed these injections. An examination of sera from sixty-five other persons inoculated with this "attenuated " virus 2 months after the. injection failed to reveal the presence of immune bodies which were thus present only in the sera of the five persons who had suffered from illnesses suggestive of yellow fever before their inoculation. It seems probable therefore, that the prophylactic injection of "attenuated " yellow fever virus given in November contained no living virus and was thus devoid of immunizing action. This conclusion is strengthened by the fact that one of those receiving the prophylactic inoculation subsequently contracted yellow fever and died (Case 4).

In the case of the sixth person suspected of having had yellow fever, it was only possible to examine the blood 3 weeks after he had been further injected

with yellow fever virus and immune serum. At that time his serum protected in a dilution of 1 in 128.

The evidence thus suggests that among Europeans the outbreak was more extensive than was at first suspected. The same appears to have been true also in the case of Africans. Four African servants, employed by G.P. (Case 4), were found to have suffered during October and November from febrile attacks with headache and vomiting. G.P.'s personal servant, who was thought to have suffered from influenza, was personally tended by his master. He and his three fellow servants all showed immune bodies to yellow fever in their bloods. In the same way an African servant in the employ of two of the suspected cases was also found to have suffered from " fever " in November. He, too, protected against yellow fever.

In order to throw further light on contact infection, sera were examined from eleven young adult Africans who either had suffered from fever during the previous year or during the same period had been in close contact with a fatal case of acute illness. Of these eleven Africans, five showed immune bodies to yellow fever in the blood serum.

Thus, including V.R., of seventeen Africans who had either suffered from undiagnosed fever during 1934, or had been in contact with a fatal case of acute illness, eleven or 64·7 per cent. protected against yellow fever. As controls to this series, bloods were obtained from twenty Africans, permanent residents of Bathurst, 18 to 40 years of age, who had not suffered from any illness during 1934 and as far as they knew, had not been in contact with any acute illness ; eight or 40 per cent. protected against yellow fever.

The figures both for adult contacts and controls are thus higher than those obtained by BEEUWKES and MAHAFFY (1934) who found that among twenty-three children from Bathurst six or 26 per cent. were positive, the youngest positive donor being 5 years of age.

YELLOW FEVER IN THE GAMBIA PROTECTORATE.

While recurrent outbreaks of yellow fever have been a feature of the history of Bathurst since its foundation 120 years ago, no epidemic of yellow fever has been recorded in the same period in the Protectorate. In 1860, however, three medical officers are said to have died from fever in the same house at MacCarthy Island after 5 days illness; and in 1911, FLOURENS (1913) records that two customs officials died in French territory on the frontier of the Gambia.

Evidence that yellow fever is certainly endemic in many villages in French Senegal, in the neighbourhood of the Gambia, was provided by the survey conducted by STÉFANOPOULO (1933) who found positive sera in Saboya in Senegal not far from the northern frontier of the Gambia.

BEEUWKES and MAHAFFY (1934) also found that of fourteen adults from Georgetown 35 per cent. were positive, while of thirty-one bloods from children

from the same town 23 per cent. were positive, the youngest positive donor being 10 years of age. These bloods were, however, co.lected from boys at the Armitage Schools, MacCarthy Island, and as their homes were widely scattered, no exact evidence was obtainab!e as to the distribution of yellow fever in the Gambia Protectorate.

In order to throw further light on the problem of yellow fever in the Protectorate, bloods were obtained from the following localities : Bakau, Brikama, Kerewan, Georgetown and Basse. The position of these villages is shown in the accompanying sketch map from which it will be seen that the Gambia forms a narrow enclave into French territory. With the exception of Bakau, all the villages are surrounded by orchard bush. Great care was taken to obtain
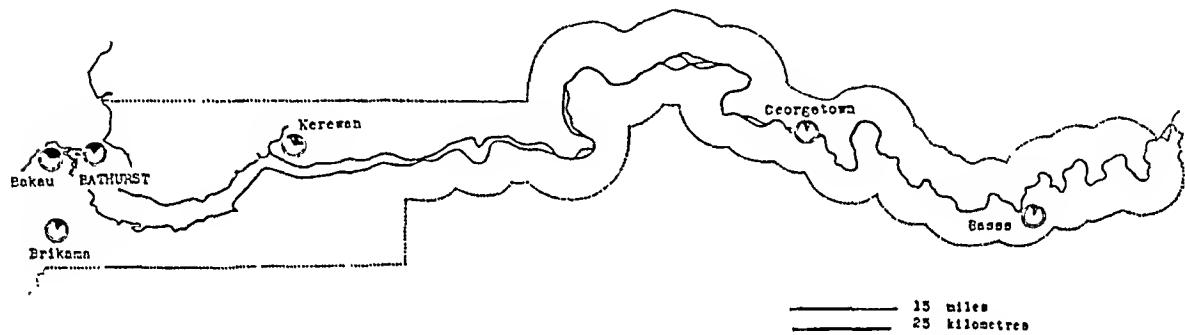
MAP OF GAMBIA.

Outer circles represent adults : inner circles represent children : black sectors indicate the proportion with protective sera.

bloods only from persons who had been born and bred in the respective towns. At least forty bloods were obtained from each town, the population being divided into four age groups, (1) under 10 years, (2) 10 to 20 years, (3) 20 to 40 years, (4) above 40 years. At the same time efforts were made to obtain bloods as often as possible from blood relations in order to determine whether there was any evidence of the infection having a familial or house distribution.

The results of the survey are shown in Table III. In all the five localities bloods were found containing immune bodies against yellow fever, the total percentage of positives varying from 20 per cent. in Basse to 33·8 per cent. in Brikama. In children under 10 years of age the highest percentages were at Bakau and Kerewan—36 per cent. Bakau, the village at Cape St. Mary, is, of course, only 7 miles from Bathurst and in daily communication with it : in addition, a number of Government bungalows are built on the cliff-edge at Cape St. Mary within 100 yards of the village, so that there are thus many opportunities for infection to pass from Bathurst to Bakau and *vice versa*. There is, however, no record of any case of yellow fever ever having occurred in Bakau.

<div align="center">TABLE III.</div>

<div align="center">YELLOW FEVER IMMUNE BODIES IN THE BLOODS OF AFRICANS FROM THE GAMBIA.</div>

Locality.	Age Groups.									Percentage Protecting.		
	Under 10 Years.			10–20 Years.		20–40 Years.		Above 40 Years.		Under 10 Years.	Over 10 Years.	Total.
	P	NP	Approximate Age in Years of Youngest Positive.	P	NP	P	NP	P	NP			
Brikama (South Bank Province)	3	11	8	1	9	5	5	4	6	21	33	33·8
Baku (Cape St. Mary)	4	7	4	1	13	5	4	3	2	36	32	30·7
Kerewan (North Bank Province)	4	7	5	3	8	4	8	1	9	36	24	27·0
Georgetown (MacCarthy Island)	2	10	7	2	12	4	8	6	4	16	33	29·0
Basse (Upper River Province)	1	9	9	2	13	2	10	5	7	10	23	20·0

<div align="center">P = protection ; NP = no protection.</div>

At Georgetown and Basse, the number of children under 10 with positive sera was small, only one, a boy of approximately 9 years of age, being found in Basse and two of 7 and 8 in Georgetown. On the other hand, the number of positive bloods in these up-river towns was high in the age groups 20 to 40 and 40 upwards. Kerewan, in the North Bank Province, was the only town in which it was possible to examine bloods from females. Of seventeen tested five were positive, three between 20 and 40 years, one between 10 and 20 years and one only under 10. No evidence could be obtained indicating any close domiciliary or family relationship between those with positive sera in any of the localities investigated.

<div align="center">MOSQUITOES IN THE GAMBIA IN RELATION TO YELLOW FEVER.*</div>

Although time did not allow of extensive collecting, it was possible to determine the presence in the Gambia of three species of Culicidae not previously

*In this connection we desire to thank Dr. F. W. EDWARDS of the British Museum (Natural History) and Mr. B. JOBLING, Entomologist to the Wellcome Bureau of Scientific Research, for their kindness in identifying the mosquitoes collected in the Gambia.

recorded from this area, *Anopheles rufipes* Gough from Keruan, *A. mauritianus* Duruty and D'Emmerez, and *Mansonia africana* Theo. from Kauur. A complete list of the Culicidae so far recorded from the Gambia is given in Table IV. The following culicine species are known to transmit yellow fever by their bites, *Aëdes aegypti* L., *A. luteocephalus* Newst, *A. vittatus* Bigot, *A. simpsoni* Theo., *Culex fatigans* Weid., *C. thalassius* Theo., *Eretmopodites chrysogaster* Graham and *Mansonia africana* Theo. In addition, the following species, while unable to transmit yellow fever by biting,. can, nevertheless, harbour the virus for considerable periods—*Aëdes irritans* Theo., *A. nigricephalus* Theo., and *Mansonia uniformis* Theo.

Eight known transmitters of yellow fever are thus present in the Gambia. The most interesting fact, however, in regard to the distribution of these vectors of yellow fever is the rarity of *Aëdes aegypt* outside Bathurst. On the Island of St. Mary, *A. aegypti* was readily found and was undoubtedly the commonest yellow fever vector present, though *Culex thalassius*, which together with *A. irritans* breeds in the innumerable crab holes, was also numerous. In the Gambia Protectorate, however, there is only one record of *A. aegypti* at at MacCarthy Island. In our own brief survey, *A. aegypti* was again absent. This does not, of course, mean that it cannot be found by intensive search, but it does suggest that possibly *A. aegypti* does not play the predominant role in transmitting yellow fever in rural areas in the Gambia.

The possibility that other mosquito vectors may play a part in transmitting yellow fever in the Gambia raises the further question whether any animals form alternative hosts for the virus of yellow fever, as is possibly the case in South America (SOPER, 1935). A certain number of bloods were obtained from monkeys caught in the neighbourhood of Bathurst, while a limited number of bloods from domestic animals were also examined for immune bodies to yellow fever. The results which have already been discussed by FINDLAY, STÉFANOPOULO, DAVEY and MAHAFFY (1936), showed that two sheep gave positive bloods while the monkey bloods were negative. In other areas in Africa—the Gold Coast, French Guinea and the Belgian Congo—positive primate bloods were obtained. It is therefore possible that in rural areas a supply of infected mosquitoes, other than *A. aegypti*, may be maintained by feeding on certain wild or domestic animals.

DISCUSSION.

The facts described in connection with the incidence of yellow fever in the town of Bathurst and in the Gambia Protectorate raise two important questions : (i) What is the explanation for the occurrence at intervals of some years of explosive epidemics of yellow fever in an urban community ? (ii) What is the explanation for the apparent occurrence of yellow fever in rural areas in the complete absence of epidemics as shown by the presence of yellow fever immune bodies in the sera of persons living in these areas ? If an answer

TABLE IV.

MOSQUITOES IN THE GAMBIA.

FAMILY CULICIDAE.

Species.	Date.	Locality.	Collector.
SUB-FAMILY ANOPHELINAE.			
Nyssorhynchus pharoensis Theo.	1902	Bathurst	Dutton
„ „	1911	Gasan	Simpson
Anopheles funestus „	1902	Bathurst	Dutton
„ „ var. *subumbrosa* Theo.	„	„	„
„ „ „ *umbrosa* Theo.	„	„	„
* „ *costalis* Loew.	1902	„	„
„ „	1911	MacCarthy Island	Simpson
„ „	1924	Bathurst	Innes
„ „	1934	„	
„ „	„	Keruan	
„ „	„	Kauur	
„ *rufipes* Gough	„	Keruan	
„ *mauritianus* Duruty and D'Emmerez	„	Kauur	
SUB-FAMILY CULICINAE			
Culex annulioris var. *gambiensis* Theo.	1902	Bathurst	Dutton
C. duttoni Theo.	„	„	„
„	1911	„	Franklin
„	1924	„	Innes
C. invidiosus Theo.	1902	„	Dutton
†*C. fatigans* Wied.	1902	„	„
†*C. thalassius* Theo.	1924	„	Innes
„	1934	„	
C. nebulosus Theo.	1929	„	Innes
C. poecilipes Theo.	1902	„	Dutton
„	1934	Basse	
C. tigripes Grandpré	1911	Bathurst	Franklin
„	1924	„	Innes
C. decens Theo.	1911	Brikama	Simpson
†*Aëdes aegypti* Linn.	1902	Bathurst	Dutton
„ „	1911	„	Simpson
„ „	1922	„	
„ „	1924	„	Innes
„ „	1934	„	
„ „	1912	MacCarthy Island	
A. irritans Theo.	1929	Bathurst	Innes
„	1934	„	
†*A. luteocephalus* Newst	1929	„	Innes
„		Bakau	„
†*A. vittatus* Bigot	1902	Bathurst	Dutton
„	1929	„	Innes
†*A. simpsoni* Theo.	„	„	„
A. nigricephalus Theo.	„	„	„
†*Eretmopodites chrysogaster* Graham	„	„	„
Mansonia (Mansonioides) uniformis Theo.	1902	„	Dutton
„ „ „	1911	Kulari	Foster
M. africana Theo.	1934	Kauur	

† Can transmit the yellow fever virus by its bite.

* Can only transmit the yellow fever virus on injection.

could be found to these questions it would go far to solve much that is now obscure in the epidemiology of yellow fever.

So far as Bathurst is concerned, there is no proof either that yellow fever is constantly present in the town or that it is introduced by shipping from other ports on the coast. The only external source of infection would thus lie in the surrounding country. All the evidence, however, points to the fact that the 1934 epidemic was of a typical urban character. There is therefore no need to invoke factors other than (1) the virus, (2) *Aëdes aegypti* and (3) the non-immune human being. The occurrence of an epidemic might conceivably be due to variation in one or more of these three factors.

In the case of certain viruses such as those of influenza and foot and mouth disease, it has been suggested that periodic variations in virulence may occur. The evidence, however, is by no means conclusive and in the case of the yellow fever virus there exists no clear indication of any periodic variation in virulence.

It is possible that periodic variations may occur in the numbers of the mosquito population. It is now known that, in association apparently with climatic cycles, there occur cycles in the population of animals as diverse as silver foxes, lemmings and gall-midges. The numbers of any one species gradually rise to a peak ; then, as a result of food shortage and disease, fall with startling rapidity. In the case of field voles (*Microtus*) in which a 3 to 4 year cycle exists it is now possible to predict with considerable accuracy the times when such population peaks are due (ELTON, DAVIS and FINDLAY, 1935). Whether mosquitoes pass through similar cycles is unknown, as experimental observations are entirely lacking : on *a priori* grounds, however, there is a strong probability that such cycles do occur.

The third factor in the genesis of recurrent urban epidemics is the presence of non-immune human beings. It has previously been pointed out that in the case of Bathurst increase of population takes place not by an excess of births over deaths, for the reverse is usually the case, but by immigration from the country. A number of these immigrants will be already immune to yellow fever but, if it be assumed that the percentage of immunes among the immigrants is less than among the urban inhabitants, while the percentage of active immunes is also less among the entrants by birth than among the departures by death, it follows that in the course of a few years the proportion of non-immunes in the urban population will gradually rise. If infection is then introduced from the country an epidemic will occur. It must be recognized that the individual carrying the infection need not necessarily be a virulent case of yellow fever : he might well be a case of inapparent infection. In this connection, it must also be remembered that many species of animals both wild and domestic which do not exhibit any symptoms of yellow fever after inoculation may nevertheless allow the virus to circulate in their blood for some days : if brought into the town during this period they would provide a source from which urban mosquitoes could become infected.

One other point in connection with the possible introduction of yellow fever from the country to the town appears worthy of further consideration. In connection with certain malaria outbreaks it has been shown that prior to their onset an immigration of anopheline mosquitoes occurs. Whether similar immigrations of aedine mosquitoes take place is as yet unknown.

From all the evidence it is suggested that yellow fever is not endemic in the town of Bathurst, but that the virus is periodically reintroduced, either by means of an infected human being, in the blood of an infected animal or possibly even by the immigration of infected mosquitoes. Whether an epidemic actually occurs will depend on the proportion of non-immunes in the population and on the stegomyia index.

To explain why no outbreak of yellow fever has ever been recorded in the Gambia Protectorate, though from 18 to 33 per cent. of the inhabitants possess immune bodies to yellow fever, requires a consideration of the divergences shown by SOPER (1935) to exist in South America between urban and jungle yellow fever.

Before discussing these divergences, however, it should be emphasized that no serious criticism has been levelled against the high degree of specificity of the mouse protection test. Of the diseases with which yellow fever might be confused clinically, malaria, blackwater fever, dengue, Rift Valley fever, epidemic catarrhal jaundice and influenza, all yield negative protection tests. BOYÉ (1935) has recently suggested on somewhat slender evidence that " red fever " (fièvre rouge du Congo) may give a positive protection test. Apart from the fact that red fever with its conspicuous symptoms has never been recorded from areas such as the Anglo-Egyptian Sudan where sera give a positive test for yellow fever, a recent observation by one of us (G.M.F.) has shown that serum from a fully authenticated case of red fever, for which we are indebted to Dr. C. C. CHESTERMAN, did not show the presence of immune bodies to yellow fever virus. There is thus little doubt that the high percentage of positive sera found in the Gambia Protectorate is evidence that infection with the virus of yellow fever has occurred.

The fact that no clinical cases have been noted may be due to a combination of circumstances such as :—

(*a*) The racial resistance of the African to yellow fever as a result of which the disease is usually mild or inapparent and the mortality rate low.

(*b*) The possibility that in certain cases immune bodies may be transmitted hereditarily from mother to child : during the period of passive immunity the child might be bitten by infected mosquitoes, thus acquiring an active immunity without clinical symptoms.

(*c*) The entire absence in African villages of any record of deaths.

(*d*) The possibility that in many African villages infection is acquired not in the house but in the fields as a result of the bites of certain non-domestic

mosquitoes. If solitary cases occurred only at irregular intervals, there would be little chance of their being seen by European observers.

Now the characteristics of jungle yellow fever, as described by SOPER (1935), are :—

(i) The disease is endemic, explosive outbreaks do not occur.

(ii) The disease is not transmitted by *Aëdes aegypti*.

(iii) The disease does not attack whole families and is contracted not in the house but in the field.

(iv) Young adults are, therefore, more likely to be attacked than small children.

(v) Immune bodies are found in the bloods of monkeys obtained from endemic areas, infection being maintained in the absence of susceptible human beings.

It is obvious that a close similarity exists between South American jungle yellow fever and the conditions existing in the Gambia Protectorate. Further observations are obviously required before it can be said that typical jungle yellow fever actually exists in Africa: in particular, evidence must be obtained that yellow fever is occurring in the complete absence of *Aëdes aegypti*. It is however certain that the epidemiology of yellow fever in Africa is far more complicated than was at one time supposed. If jungle yellow fever, transmitted by non-domestic mosquitoes, actually exists, preventive measures of value in combating urban yellow fever are of little avail. Personal prophylaxis by artificial immunization is at present all that can be attempted. In the Gambia this has been carried out since the greater part of the European population has now been actively immunized. The results of this immunization will form the subject of a further communication.

So far as urban outbreaks are concerned, the reduction to and maintenance of the mosquito index at zero is sufficient to guard against the possibilities of reintroduction of the virus.

CONCLUSIONS.

1. The epidemic of yellow fever that took place in Bathurst in 1934 is described. Evidence is brought forward to suggest that the cases actually diagnosed were but a part of those that actually occurred.

2. Although yellow fever epidemics have never been described among Africans living in the Protectorate, a survey of the Gambia shows that in a number of villages from 20 to 33 per cent. of the inhabitants possess immune bodies to yellow fever.

3. A list of the culicine mosquitoes recorded from the Gambia is given.

4. The urban type of yellow fever seen in Bathurst is contrasted with the rural type found in the Protectorate. The possibility that the rural type of yellow fever in Africa is similar to the jungle yellow fever of South America is discussed.

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THE BLOOD CHEMISTRY OF NORMAL SOUTHERN RHODESIAN NATIVES.

BY

WILLIAM STONE, M.A., B.Sc.*

From the Public Health Laboratory and Pasteur Institute, Salisbury, Southern Rhodesia.

In the past, normal blood chemistry values obtained in Europeans were used in the interpretation of results obtained in the blood of Bantu patients. That this was not always justified was shown by the discrepancy in biochemical and clinical findings often found in this laboratory. In this connection, a very low calcium figure, often below 7 mg. Ca per 100 c.cm. plasma, was observed, without any clinical symptoms whatsoever. Dr. F. W. Fox, of the South African Institute for Medical Research, Johannesburg, reports that he got definitely low blood calcium values in a series of presumably healthy boys, and believes that the same has been found by other observers. (Personal communication.)

As far as we were able to determine, there are no references in the literature to normal Bantu blood chemistry : Dr. Fox was also unable to supply us with any references. Therefore, Dr. W. K. Blackie, Director of this laboratory, suggested that this subject be investigated.

Fifty normal, adult, male natives were selected for the study. Thirty were picked at random from boys working in town and twenty were members of the British South Africa Police. Of these twenty, ten were recruits (less than 3 months in the Service) and the other ten had been in the Service for a year or more. The determinations made on their blood were sugar, chlorides, non-protein nitrogen (N.P.N.), urea, cholesterol, plasma calcium, and plasma inorganic phosphorus.

METHODS.

1. *Blood Sugar* : The method of Folin and Wu, as described by Hawk and Bergheim (1927).

2. *Chlorides* : The method of Whitehorn. (Hawk and Bergheim.)

3. *Non-Protein Nitrogen* : The method of Folin and Wu. (Hawk and Bergheim).

4. *Urea*: The method of KOCH and McMEEKIN, with aeration and nesslerization. (HAWK and BERGHEIM.)

5. *Cholesterol*: The method of KAMLET (1934).

KAMLET found that cholesterol was quantitatively extracted from blood dried on filter paper, in 2 hours at room temperature.

0·2 c.cm. of blood was spread evenly on a 7 cm. filter paper (Whatman 30). It was dried in an incubator at 37° C., for about one half-hour. The paper was then rolled compactly, folded in quarters, and deposited in a large test-tube. 10 c.cm. of chloroform were pipetted in, and the paper completely covered. The tube was stoppered and let stand for 2 hours. At the end of that time, 5 c.cm. of the extract was treated with 2 c.cm. of acetic anhydride and 0·2 c.cm. concentrated H_2SO_4. It was then compared with 5 c.cm. of a chloroform solution of cholesterol (containing 0·05 mg. per c.cm.) treated in the same way. Before comparison, both tubes were left in the dark for 15 minutes.

6. *Calcium*: CLARK-COLLIP modification of the KRAMER-TISDALL method. (HAWK and BERGHEIM.)

7. *Inorganic Phosphorus*: The method of FISKE and SUBARROW. (HAWK and BERGHEIM).

RESULTS.

A comparison of the various groups is made in the table opposite :—

Blood Sugar.

The values obtained suggest that the normal blood sugar of natives is somewhat lower than the accepted European average. In the majority of the natives this fell between 70 and 100 mg. per 100 c.cm. blood (80 per cent.) Six were below 70 mg. (12 per cent.) and four were above 100 mg. (8 per cent.). The suggested normal range would be 70 to 100 mg./100 c.cm. blood. All blood sugars were done immediately after drawing the blood.

Chloride.

Chloride values are also on the low side of normality, probably due to excessive sweating in this tropical climate. 86 per cent. were between 260 and 300 mg. Cl/100 c.cm. blood. This is the suggested normal value. One (2 per cent.) was below 260, and 6 (12 per cent.) above 300. Chloride determinations on Europeans also show low normal values, an average of 275 being obtained for ten determinations.

Non-Protein Nitrogen and Urea.

These figures would naturally depend on the protein intake of the individual. This varies greatly among the natives in town, house servants getting more meat as a rule than natives employed in industry. This accounts for the variations noted.

TABLE I.

Blood Constituents Mg/100 c.cm.	Normal White.	Ordinary Natives (30).		Native Police.							Total Native (50).	
				Old Members (10).		Recruits (10).		Total (20).				
	Range.	Mean.	Range.	Mean.	Range.	Mean.	Range.	Mean.	Range.		Mean.	Range.
Sugar (Folin & Wu)	80–120	88·6	62–117	88·2	61–111	84·6	72–98	86·4	61–111		86·5	61–117
Chloride As Cl	270–320	286·4	250–330	274·5	265–300	280·0	260–295	277·3	260–300		282·8	250–330
N.P.N.	25–50	35·2	24–43	24·2	20–38	27·9	18·6–38	26·0	18·6–38		31·5	18·6–43
Urea	15–40	24·9	16–31	20·4	18–30	20·4	12–30	20·4	12–30		23·1	12–31
Cholesterol	150–200	126·8	75–190	150·9	145–165	105·1	87–125	128·0	87–165		127·1	75–190
Calcium As Ca	9–11	9·11	6·0–11·0	9·40	7·5–11·0	8·94	6·8–10·8	9·17	6·8–11·0		9·14	6·0–11·0
Inorganic Phosphorus As P	3–4	2·92	1·65–4·10	3·32	2·88–3·84	2·83	2·07–3·52	3·08	2·07–3·84		2·98	1·65–1·10

Notes.—

1. Ca and P values are for plasma.
2. Range figures are extreme variations. Suggested normal ranges will be given later.
3. Normal white values taken from *Practical Physiological Chemistry* (HAWK & BERGHEIM, 1927), and *Chemical Methods in Clinical Medicine* (G. A. HARRISON, 1930).

c

N.P.N.

94 per cent. of the natives fell between 20 and 40 mg./100 c.cm. of blood. This is the normal range suggested.   Two (4 per cent.) fell below 20, and one (2 per cent.) above 40.

Urea.

90 per cent. fell between 15 and 30 mg./100 c.cm. of blood (normal range). Three (6 per cent.) were below 15 mg. and two (4 per cent.) above 30.   The diet of the native police will be discussed later.

(See Summary on p. 172 for effect on blood urea.)

Cholesterol.

The figures obtained show an extremely wide variation. This, in the ordinary natives, who were done first, ranged from 75 to 190 mg./100 c.cm. blood. A study of the Native Police figures, however, suggested a possible explanation.   It will be noticed that those who have been in the service for a year or more show a normal average, 150·9 mg./100 c.cm. blood, with a very small variation.   The recruits, on the other hand, are definitely low, with an average of 105·1, and a somewhat larger variation.

DUFF (1935), in a review on experimental cholesterol arteriosclerosis, provides us with the following information.

" The effect of cholesterol feeding on the cholesterol content of the blood varies in different species of animals. Dogs, cats and other carnivores, and monkeys respond to single doses, and also to continued feeding with only a moderate and transient increase in blood cholesterol.   In rabbits (and guineapigs) an enormous increase in the cholesterol content of the blood can be produced by feeding on cholesterol or egg-yolk."

" The rabbit is an herbivorous animal, while man is omnivorous. Cholesterol is completely lacking in vegetable diets, although phytosterol is present in comparatively small amounts. Cholesterol is present in varying amounts in the diet of omnivorous animals.   In man, a large quantity of cholesterol in the diet has only a relatively slight and temporary effect on the level of cholesterol in the blood, owing apparently to the facility with which cholesterol is disposed of by the human mechanism.   The normal blood cholesterol content in man is about twice that of the rabbit."   (Therefore, the rabbit blood cholesterol can be assumed to be about 75 to 100 mg. per 100 c.cm.)

" Cholesterol is contained in the human diet, being in considerable quantities in eggs, milk, butter and meat. The amount ingested undoubtedly varies considerably between persons, and from time to time in any single person, but the variations in the cholesterol content of the diet have never been shown to have any significant effect on the cholesterol content of the blood. Furthermore, the deliberate addition of cholesterol to the food of normal subjects does not produce a sustained hypercholesteremia.   The effect on the level of the blood cholesterol is relatively slight and transitory.

" Not only is cholesterol present in greater concentration in human plasma (than in the rabbit), but it normally stands at a level not far from the point of saturation."

LEATHES (1925), says that cholesterol is present in the fat of tissues to the extent of 5 to 25 per cent. of the amount of fatty acids, though almost absent from adipose tissue. The highest proportion of cholesterol (and its esters) is found in nervous tissue, then lung, kidney, liver, pancreas, heart, testicle and muscle.

STARLING (1933), says that cholesterol is undoubtedly synthesized in the animal body.

Inquiry was made as to the diets of natives, including police. The information obtained also has a bearing on the calcium and phosphorus figures, to be discussed later.

Natives living in a kraal, which is in good condition, not over-stocked, and where hunting is allowed (as in the tsetse fly belt) have a good and varied diet. It consists mainly of : Kaffir corn, brown rice, Kaffir beer, various roots, fruit, dried and green vegetables, all sorts of animals, from game to insects, fruit wine, honey beer, honey cakes, sugar cane, peanuts, yams, maize, sour milk, etc. Obviously such a diet contains all the necessary food constituents.

However, when the areas become overstocked and poor, all the vegetable matter and fruit disappear, meat is reduced to almost zero (the natives will not eat their cattle), and the main food is derived from maize meal. This is often obtained from traders, and may have most of its valuable constituents milled out of it. Under such conditions, malnutrition is a very common occurrence. It is obvious that in these congested areas, the cholesterol intake is reduced to a very low minimum. If a child has been brought up to manhood by means of such a diet, it is quite conceivable that his blood cholesterol will be near the level of the herbiverous animals, such as the rabbit. All of the recruits to the British South Africa Police (B.S.A.P.) at Salisbury come from such congested areas. Their blood cholesterol figures parallel those of the rabbit. If now, these natives are put on a diet containing a good deal of cholesterol, it is quite possible that their blood cholesterol would rise to the normal European value. The rise would not go beyond that, because as DUFF has pointed out, the human mechanism would excrete any excess.

The town native police buy their own food. A check-up has shown that this consists of tea, sugar, bread, maize meal, Kaffir beer, vegetables, peanuts, sour milk, and about 1 lb. of meat a day. This meat consists mainly of kidneys, testicles, pancreas, liver, lungs and heart. Brain is consumed as a remedy for illness. They are fond of these organs, and they are cheap. These are the very parts of the animal which LEATHES states are the richest in cholesterol. It is, therefore, likely that individuals who have low blood cholesterol due to faulty diet, can be brought up to normal by the administration of foods rich in cholesterol. The old native police show a normal figure.

The distribution of cholesterol figures among the thirty ordinary natives was as follows :—

75-99 mg./100 c.cm. blood	5	140-159 mg./100 c.cm. blood	3
100-119 ,,	10	160-179 ,,	5
120-139 ,,	5	180-190 ,,	2

It is apparent, then, if the conclusions drawn above are correct, that the 33 per cent. of the natives who fall within the normal range have had either good

diets during their lifetime, or have been exposed to a cholesterol-rich diet for some time. An attempt will be made to follow the cholesterol content of the blood of recruits, over a period of time, on such a diet.

In view of the above, no normal range will be suggested. The European range will be satisfactory under good dietary conditions.

Calcium and Inorganic Phosphorus.

As these two elements go hand in hand in nutrition and metabolism, they will be treated under one head.

The variation in *calcium* was as follows :

6·0-6·9 mg. Ca/100 c.cm, plasma	3	9·0- 9·9 mg Ca/100 c cm plasma	15
7·0-7·9	„	4	10·0-10·9	„	13
8·0-8·9	„	12	11·0	„	3

The variation in *phosphorus* was as follows :—

1·50-1·99 mg. P/100 c.cm. plasma	$\overline{2}$	3·00-3·49 mg. P/100 c.cm. plasma	14
2·00-2·49	„	7	3·50-3·99	„	8
2·50-2·99	„	17	4·00-4·10	„	2

These figures indicate a serious dietary deficiency in some of the natives. The native police figures indicate that the more varied diet helps to raise these figures, as well as that of cholesterol.

Ten calcium determinations on Europeans gave a mean of 9·05 mg. Ca/100 c.cm. plasma ; and eight inorganic phosphates on the same people, 2·86 mg. P/100 c.cm. plasma. The range for Ca was 7·2-10·9, with four 8·0 or below ; and for P, 2·56-3·70, with six out of eight below 3·00.

Apparently, then, calcium and phosphate deficiency is not confined to natives. As Europeans normally have a varied diet, there must be a shortage of these elements in the local food supply. The main source of Ca and P for the growing child is milk. A sample of pasteurized milk was obtained locally. Its analysis was as follows : Ca, 0·116 per cent. ; P, 0·077 per cent.

SHERMAN (1932) gives for milk : Ca, 0·120 per cent. ; P, 0·093 per cent.

BODANSKY (1934) gives 0·144 per cent. Ca in milk.

HAWK and BERGHEIM (1927) give 0·160 per cent. Ca in milk.

It is easy to see that the milk is definitely low in phosphorus, and not too good in calcium. This would also affect local milk products such as cheese.

Mealie (maize) meal (ground from the whole maize and not refined) analyzed as follows : Ca, 0·0096 per cent. ; P, 0·190 per cent.

SHERMAN gives for dried maize : Ca, 0·021 per cent. ; P, 0·376 per cent.

Fox (1936) states that " the composition of mealie meal varies quite appreciably according to the manner in which it is prepared. However, making the rather unlikely assumption that it has been prepared by the most conservative treatment of the whole grain, we have a meal as follows : Ca, 0·014 per cent. ; P, 0·25 per cent.

Both the Rhodesian and the South African mealie are then definitely low in calcium and phosphorus.

The average native on rations gets 2 lb. of mealie meal per day, and 2 lb. of meat per week. Average meat, according to SHERMAN, contains 0·012 per cent. Ca, and 0·22 per cent. P. Assuming these figures to hold for Rhodesian meat (unlikely, because of the lack of Ca and P in the soil, and, therefore, in the grass which the cattle eat) then this native will consume a total of 0·10 gm. of Ca per day, and 2·00 gm. of P per day. According to SHERMAN, the allowance for adult maintenance is 0·68 gm. Ca per day and 1·32 gm. P. Unless the native supplements his diet with Ca-rich foods, which is impossible in the crowded reserves, and extremely unlikely in town, considering the salaries paid to the natives, he runs far below his daily Ca requirement. This undoubtedly accounts for the low calciums seen in the town natives and the recruits.

TABLE II.

SUGGESTED NORMAL RANGE FOR NATIVES AS COMPARED WITH WHITES.

Blood Constituents Mg/100 c.cm.	Native.	White.
Sugar	70–100	80–120
Chloride As Cl	260–300	270–320
N.P.N.	20–40	25–50
Urea	15–30	15–40
Cholesterol	150–200	150–200
Calcium As Ca	9–11	9–11
Inorganic Phosphorus As P	3–4	3–4

Note.—Ca and P values are for plasma.

It is also very likely that in the crowded reserves, the phosphorus intake falls below the daily requirement. This may also happen if the town native gets refined maize meal. SHERMAN states that refined corn (maize) meal contains only half the amount of phosphorus that dried maize does. The calcium is reduced by 14 per cent.

The normal European range for calcium and phosphorus should be adhered to, but some allowance must be made in regard to low blood calcium and tetany. None of the natives with calciums below 7·0 showed any clinical signs. Perhaps

a low calcium diet during a lifetime, or even for several generations, may develop resistance to tetany in the native.   This should be investigated.

Fox (personal communication) reports a series of low blood calciums in apparently healthy natives, but gives no reason for this.

SUMMARY.

The blood chemistry of fifty normal Rhodesian natives was investigated. The tests made were sugar, chlorides, non-protein nitrogen urea, cholesterol, calcium and phosphorus.   Comparison of various groups showed :

1. The possibility of bringing low blood cholesterol figures to the normal value by feeding with cholesterol-rich foods.

2. The probability that low blood cholesterol can be caused by a lack of sufficient cholesterol in the diet.

3. A serious deficiency in calcium intake, with resultant low blood calciums. This is probably due to the following factors :

(*a*) A lack of Ca in the soil, and, therefore, in local foods.

(*b*) A lack of calcium-rich foods in the average native dietary.

4. A low blood phosphorus in some cases, but not to the same extent as in calcium.

5. Blood sugars and chlorides tend to a low European normal, while N.P.N.'s and ureas are for the most part mid-normal.   The low ureas seen in some of the police are probably due to a large consumption of Kaffir beer, which acts as a powerful diuretic.

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POLYNUCLEAR COUNT OF THE ALOUITES.

BY

WILLIAM M. SHANKLIN*

From the Department of Histology, School of Medicine, American University of Beirut, Beirut, Syria.

INTRODUCTION.

ARNETH (1904) has demonstrated that in patients suffering from active infection, the number of mature fully segmented granulocytes in the peripheral blood is relatively decreased, due to an absolute increase in the number of younger cells supplied from the bone marrow. The criterion on which ARNETH based his conclusions was the degree of segmentation of the polymorphonuclear nucleus. Although infection provides a strong stimulus in modifying the percentage of neutrophiles with a segmented nucleus (polynuclear cells), the influence of such factors as race, locality, occupation and quality of water and food may conceivably play fundamental roles in determining the " normal " blood picture, but their effects have not been adequately elucidated.

While making anthropological measurements on the Alouites or Nusariyyeh, a branch of the Shiite Moslems dwelling in North Syria in the state of Latakia, the writer had the opportunity to obtain blood smears from a representative sample of the population of certain of the Alouite villages.

MATERIALS AND METHODS.

DODD (1934) in his sociological studies on the Alouites states that 83 per cent. of them are farmers. In the Syrian population, next to the desert Bedouin, the Alouites have been least influenced by modern conditions. The writer confirms the statistical data of DODD (1934) that the simplest rules of sanitation are neglected, and that, although agriculturists, many of the Alouites suffer from a poorly balanced diet. Competent physicians rarely visit the Alouite villages ; and, due to ignorance and natural inertia, dysentery, typhoid and malaria are severe, particularly in the villages of the plain. The physical anthropology of the Alouite females has recently been described by SHANKLIN and IZZEDDIN (1936).

During a period of four weeks, the physician working in Deir Mama with the American Junior College Village Welfare workers gave free treatment to the inhabitants. Out of a population estimated at 1,000, a total of 391 persons came for treatment. The distribution of the diagnoses is as follows : malaria

109, trachoma 85, sore eyes 82, constipation 41, wounds 32, headache 14, abscesses 7, indigestion 6, dysentery 6, sore throats 3, earache 3, ulcers 3, tonsillitis 2, enlarged cervical glands 1, sciatica 1.

The polynuclear counts are reported on 323 Alouite males and females ranging in age from 6 to 60 years. Of the Alouite total, 96 subjects dwell in the plain and 136 live in the mountain village of Deir Mama. These counts were made on the general population and no attempt was made to eliminate diseased persons, although no acutely ill persons were included in the series. These counts can be considered the normal for the district.

The films of blood were made from the finger tip, after which the slide was fixed in methyl alcohol and stained with Leishman stain. All counts were made by a skilled haematological technician, strictly in accordance with the procedure of COOKE and PONDER (1927). One hundred polymorphonuclears were counted for each subject.

The polynuclear index was determined by multiplying the total number of cells in each class by the number of the class, summing the results and dividing by the number of cells counted.

RESULTS.

The polynuclear count for the plain and mountain dwellers combined was as follows below :

	I.	II.	III.	IV.	V.	Mean Index
Lowest count	96·00	4·00	0	0	0	1·04
Average count	58·93	35·42	5·04	0·56	0·08	1·48 ± 0·040
Highest count	32·00	52·00	16·00	0	0	1·84

The inhabitants of the mountain village Deir Mama appear to be healthier than the people living on the plain, hence the data on the two groups are also presented separately.

	I.	II.	III.	IV.	V.	Mean Index.
Mountain dwellers' count	57·30	37·40	4·80	0·40	0·10	1·49
Plain dwellers' count ..	61·20	32·70	5·20	0·80	0·10	1·46

The individual counts are not presented in tabular form but by a frequency polygon (p. 175) to show the Alouite distribution as compared with the Iraq counts of Kennedy (1935), and the British normal counts of KENNEDY (1933).

A study of the differential count (100 cells counted) for 235 Alouites gives the following distribution : neutrophiles 57·71 per cent., lymphocytes 31·27 per cent., monocytes 5·75 per cent., eosinophiles 5·39 per cent., basophiles 0·02 per cent., transitionals 0·02 per cent., and myelocytes none. The differential counts of the people of the plain and those of the mountain are reported together as there is no significant difference.

COMPARATIVE DATA.

In order to appreciate the significance of the marked shift to the left in the Alouite population summaries of normal counts on other racial groups are included on page 175.

Heavy line - 323 Alouites
Broken line - 121 Iraq nationals
Thin line - 90 Scotch normals

FREQUENCY POLYGON.
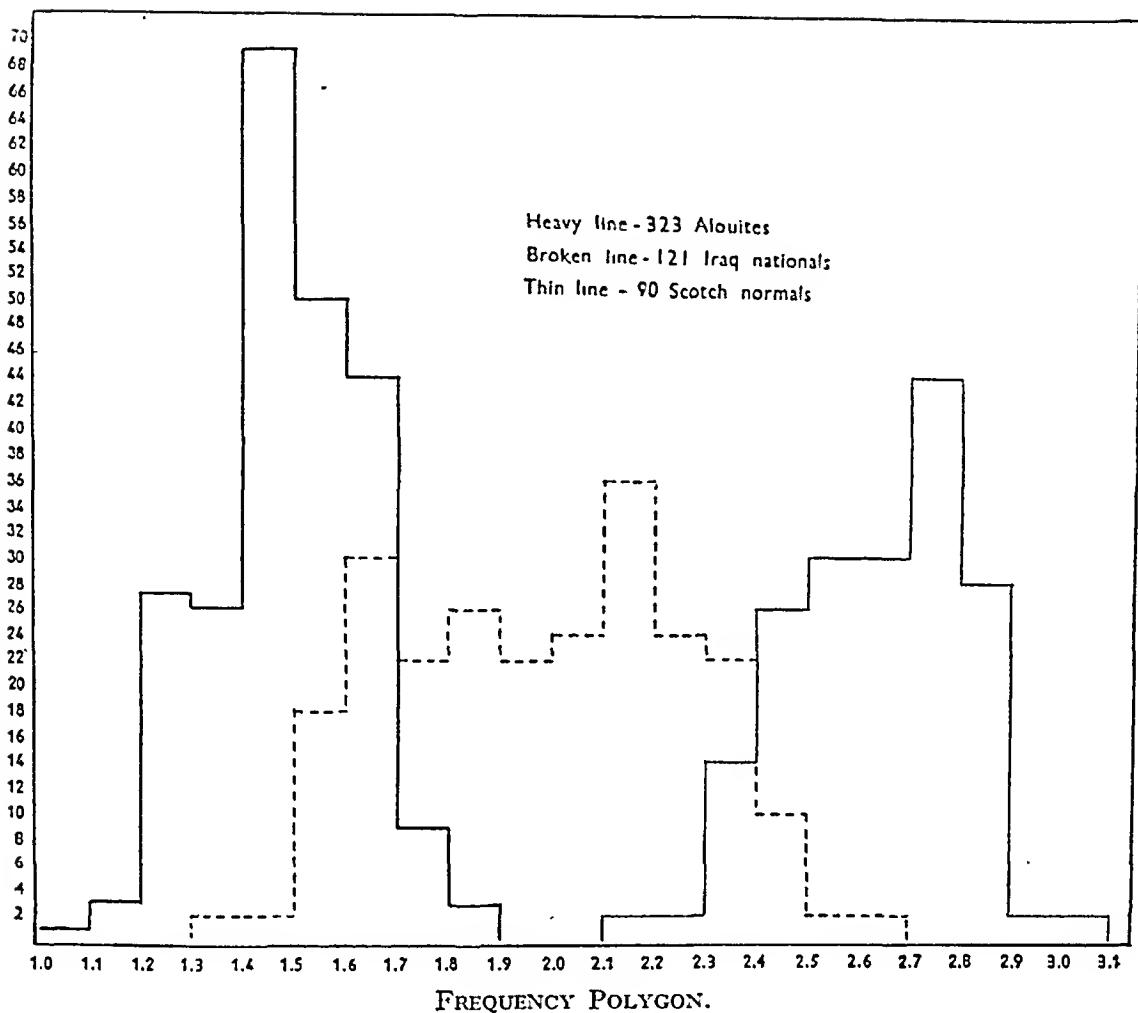
COOKE and PONDER (1927) give the following figures for English inhabitants who have been subjected to a very rigid medical examination. The counts of COOKE and PONDER are :

	I.	II.	III.	IV.	V.	Mean Index.
Lowest count	15	34	40	11	0	2·47
Average count	12	25	44	15	4	2·74
Highest count	9	24	47	17	3	3·11

KENNEDY (1933) who studied ninety Scotch males and females attributes their slightly lower polynuclear index to a less rigid medical examination than that given by COOKE and PONDER. The figures of KENNEDY are :

	I.	II.	III.	IV.	V.	Mean Index.
Lowest count	30	37	18	14·5	0·5	2·175
Average count	13	30	43	10	1	2·62
Highest count	10·5	22	39·5	28	4	3·05

ABELS (1934) found the normal index for 100 inhabitants of New York was lower than the values for the English and Scotch normals. ABELS's counts are :

	I.	II.	III.	IV.	V.	Mean Index.
Lowest count	15	59	24	1	1	2·14
Average count	12	54	27	6	1	2·30
Highest count	9	40	38	12	1	2·56

Polynuclear counts for normal persons living in widely separated areas have been reported by MACLEOD (1935) : Australia 2·64, Wigan (England) 2·61, Florida 2·58, South Africa 2·44, New York 2·44, Japan 2·34, China 2·33, and Greece 2·31. MACLEOD concludes his figures indicate that definite differences exist between the means of various localities though the mean for each locality is quite constant.

KENNEDY (1935) studied the polynuclear count of 121 inhabitants (Kurds, Dulaini and Jews) of Iraq, eliminating those obviously diseased. KENNEDY's two sets of Iraq figures are :

	I.	II.	III.	IV.	V.	Mean Index.
Average count	31	46	19	2	2	1·98
Average count	31	42	25	2	0	

As KENNEDY remarks, these counts show a marked shift to the left. As a matter of fact, the Iraq average of 1·98 is far lower than the lowest value given by MACLEOD (2·31 for Greece) in his world-wide study.

Still more striking than the findings on the Iraq population are the recent studies by KENNEDY and MACKAY (1935) on British airmen stationed near Baghdad, for these healthy airmen have a polynuclear index slightly lower than that of the people of Iraq themselves. These studies re-emphasize the effect of environment on the polynuclear count. The counts for British airman are :

	I.	II.	III.	IV.	V.	Mean Index.
Average count	30	49	18	3	0	1·94

The investigations of PAI (1935) show that the Chinese and British in Moukden tend to have similar polynuclear counts ; whereas Chinese in England tend to have counts more like those of the English.

DISCUSSION.

The outstanding finding of the study on the Alouites is the lowest mean polynuclear index (1·48) that has been reported for any racial group. The next lowest polynuclear index (1·98) reported is that for the Iraq population by KENNEDY (1935). It was the writer's expectation that the polynuclear index of the Alouite mountain dwellers from Deir Mama would be much higher than the Alouites living in the plain because malaria is far more prevalent among the latter group. However, the polynuclear index difference between the two groups is practically negligible.

The investigations of ARNETH (1904) have forcibly directed our attention to the sharp increase in the polynuclear leucocytes in Classes I and II and the decrease in Classes III, IV, V in patients suffering from a variety of diseases. However, it is not necessary to discuss the effect of different diseases on the polymorphonuclear count, for the subject has been treated in extensive works by COOKE and PONDER (1927), SCHILLING (1929), and by others in numerous shorter papers.

Malaria alone may account for the low index for most of the plain dwellers, but for the inhabitants of Deir Mama other causes must be sought. It is questionable whether the trachoma and sore eyes found in 167 cases play an important role in influencing the polynuclear index ; the same is true for the thirty-two cases of wounds, many of which were of a minor nature. Concerning such diseases as gonorrhea and syphilis there are no reliable data. The fact that such cases were not reported by the camp physician rather indicates they are not very prevalent ; however, along the Euphrates River, HUDSON (1932-33)* reported 257 cases of syphilis (bejel) among Bedouins, and 263 cases among natives of Deir-ez-Zor out of a total of 3,000 consecutive hospital admissions.

DODD (1934) has called attention to the low incidence of tuberculosis among the Alouites of the plain and attributes it to the strong sunlight, there being more than 300 cloudless days in the year, and the great amount of time spent outdoors. Also, as pointed out by DODD (1934), hookworm, so common in some parts of the Lebanon and Syria, has not reached the Alouite villages. Although typhoid fever and dysentery are prevalent at times among the Alouite villagers, there were very few cases at the time these polynuclear studies were made. According to the investigations of YENIKOMSHIAN and BERBERIAN (1934), helminthiasis is prevalent in many of the villages of Syria. Smallpox is kept in check by the active campaign of the government. It does not appear that the known diseases in Deir Mama can account alone for the low polynuclear index, but, as pointed out by KENNEDY (1935) in discussing the low Iraq index, diseases that may not prevent a man from doing his daily work may cause a considerable deviation of the polynuclear count.

The effect of a tropical or subtropical climate needs to be taken into consideration in polynuclear count studies, for CHAMBERLAIN and VEDDER (1911) found a marked shift to the left (by the original Arneth method) in Filipinos and a slight movement in the same direction for American soldiers resident in the Philippine Islands. MACFIE (1915) found a shift to the left in Europeans living in West Africa. BREINL and PRIESTLEY (1915) attribute the shift to the left in native adults and healthy white school children of New Queensland to climatic influences. The recent findings of KENNEDY and MACKAY (1935) on British airmen stationed in the Baghdad area are significant because these men, who are subjected to very rigid medical examinations, have polynuclear counts

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* For a discussion of the relationship of syphilis, bejel and yaws the reader is referred to this and other papers by HUDSON.

exacerbations : in both recovery is not accompanied by immunity to reinfection : and both are amenable to treatment with synthetic aromatic compounds, specially those of arsenic. From the serological standpoint the interest in the two infections is that in both there is evidence that the infecting parasite is able to readapt itself—or to vary serologically—several times in the body of one host without any loss of pathogenicity in the variants. It may be said, on the whole, that the animal body is incapable of ridding itself of an infection with a pathogenic trypanosome, although an antibody reaction is manifest and lysins of low titre to many serological variants of the trypanosome appear one after another in the host's serum as the infection proceeds. In relapsing fever the animal host does usually rid itself of the infection, but only after lysins of relatively high titre for several serological variants of the spirochaete have developed one after the other.

So far as I am aware it has not yet been shown that serological variation of this kind in one host is a characteristic of bacterial infections, although that may depend merely upon the limits of present-day technique. In the realm of virus disease there is even less evidence that the infecting agents can readapt themselves serologically and remain pathogenic in the course of one infection.

I propose to give a short summary of the work which has been done on the serology of relapsing fever and trypanosomiasis, and then to return to the question of variation, for in protozoal and allied infections it appears likely that the intractable type of the infections depends upon the fact that the invading parasite is an entity which is as adaptable as the host's body cells which attempt to destroy it.

Relapsing Fever.

In 1868 in Silesia OBERMEIER (1873) saw a spirochaete in the blood of patients suffering from relapsing fever, and five years later when he found it again in cases in Berlin he published the observation. This spirochaete, *Treponema recurrentis*, was the first micro-organism to be associated definitely with disease in man and it was naturally a great source of interest to the early immunologists. It remained a source of much interest until the beginning of this century, when the pure culture technique of bacteriology was becoming established. Thus METCHNIKOFF (1887), studying leucocytes at the crisis of an attack of relapsing fever, saw macrophages ingesting the spirochaetes and claimed it as proof of the cellular theory of immunity. Later, GABRITCHEWSY (1896), mixing immune relapsing fever serum with living spirochaetes saw the latter disappear into unrecognisable granules and claimed the lytic action of the immune serum as support for the theory of humoral immunity.

In the beginning of this century these theories of immunity were reconciled by the work of ERHLICH and others, and the evolution of the aromatic arsenic compounds provided a cure for relapsing fever which was astonishingly efficient.

Further, the difficulty of growing pathogenic spirochaetes outside the body was not overcome, and the interest in relapsing fever waned as it was driven back in Europe before the advance of sanitation.

During the War, and the post-war refugee period, interest in relapsing fever revived with its reappearance in many parts of Europe and interesting papers belong to this period. JANCSÓ (1918) observed that the spirochaetes of an attack of relapsing fever differed serologically from those of the relapses. In 1925, in India, CUNNINGHAM (1925) showed that at each relapse in any one animal the spirochaete is of a new serological type : he also demonstrated the alternation of types which occurs when the infection is passed on at a relapse to a new animal. Since then little that is new has been contributed to the serological study of relapsing fever. The writer (RUSSELL, 1931), working in the Gold Coast with the local louse-borne relapsing fever and *Cricetomys gambianus*, the pouched bush rat, as an experimental animal, confirmed CUNNINGHAM'S results. It was concluded that *T. recurrentis* had the capacity of producing several serological variants in one host, each variant being equally pathogenic. The limited number of variants which is detected in any epidemic was explained by the alternation of variants which occurs when the parasite reaches a new host who is not already immune to any variant : this alternation tending to keep certain variants predominant.

CHART I.

1879
March   N.P. Hindoo ward attendant.
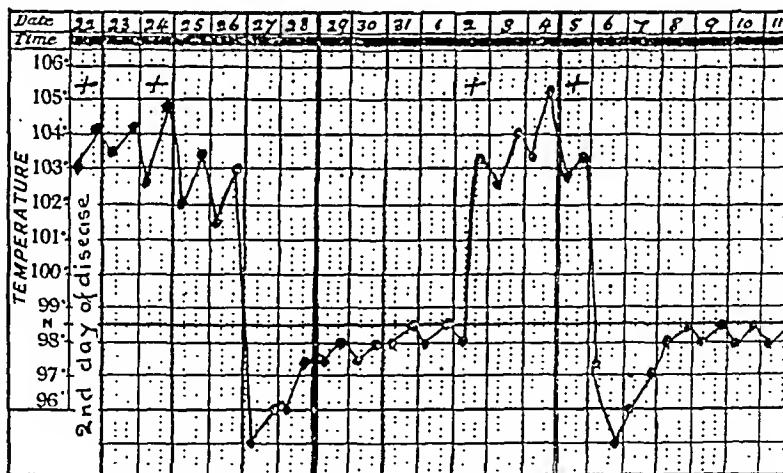
CASE OF RELAPSING FEVER.
(From " Spirillum Fever " by VANDYKE CARTER, *Med. Chir. Trans.* London lxiii.)

Chart I is included here to remind the reader of the clinical course of relapsing fever. It appears at first sight an ideal disease in which to study immunity. The acute stage of fever with parasitaemia, the crisis associated with the disappearance of the circulating spirochaetes and the appearance of a high

titre antibody, the inevitable relapse or relapses are all so well defined as to be dramatic. Chart II gives the details of the ordinary course of the fever in a

CHART II.

		DAYS→ 5 10 15 20 25 30 35 40 45 50 55 60
1	C. gambianus. 100 22·9·29 0·75 cc BLOOD FROM BRIAMA	A B a± b±
2	C. gambianus. 102 27·9·29 0·75 cc BLOOD FROM C.g 101	a± a+ b± c±
3	C. gambianus 103 30·9·29 0·5 cc BLOOD FROM C.g 102	a± b± c–
4	C. gambianus. 105 5·10·29 0·5 cc BLOOD FROM C.g 6 (FROM 103)	a+ B a± b± b+ c–
5	C. gambianus. 106, 9·10·29 0·5 cc BLOOD FROM C.g 105	a± b± c±
6	C. gambianus 108, 14·10·29 0·5 cc BLOOD FROM C.g 107	a+ a± b+ b± c±
7	C. gambianus 109 17·10·29 0·5 cc BLOOD FROM C.g 108	B b+ c–
8	C. gambianus 110 19·10·29 0·5 cc BLOOD FROM C.g 109	A a+ b± a± b+ c±
9	C. gambianus 114 29·10 29 0·75 cc BLOOD FROM C.g 113	A 1 spirochaete seen a± b c–
10	C. gambianus 115, 1·11·29 0·75 cc BLOOD FROM C.g 114	a+ a± b± a± b± c±
11	C. gambianus 118, 10·11 29 0·5 cc BLOOD FROM C.g 117	A a+ a± b± a± b± c±
12	C. gambianus 121 19·11·29 0·75 cc BLOOD FROM C.g 120	A a+ a± b± a± b± c±
13	C. gambianus 123 23·11·29 0·5 cc BLOOD FROM C.g 122	A a± a± b± a± b± c± 2 RELAPSES
14	C. gambianus 124 25·11·29 0·5 cc BLOOD FROM C.g 123	A a± b+ c–
15	C. gambianus 125, 28·11·29 1·0 cc BLOOD FROM C.g 124	A a+ B b+ b± c–
16	C. gambianus 36 1·12·29 a few months old. 0·5 cc BLOOD FROM C.g 125	a+ b± c+ 2ND RELAPSES
17	C. gambianus 37 1·12·29 a few months old 0·5 cc BLOOD FROM C.g 125	a+ b– a± b± c±
18	C. gambianus 127, 4·12·29 1·0 cc BLOOD FROM C.g 36	a± b± c–
19	C. gambianus 128 6·12·29 0·5 cc BLOOD FROM C.g 127	a± b– c– ATTACK ONLY
20	C. gambianus 131, 11·12·29 2·0 cc BLOOD FROM C.g 129	a± b± c–
21	C. gambianus 134 19·12·29 0·5 cc BLOOD FROM C.g 133	a+ c–
22	C. gambianus 144 14·1·30 0·5 cc BLOOD FROM C.g 143	a+ SUPPRESSION b± c± OF ATTACK

RELAPSING FEVER IN *C. gambianus*.

■ = spirochaetes present.   A and B = type of spirochaete
▌ = spirochaetes numerous.   a, b and c = type of serum.
                    ↑ = day on which serum tested.

series of *C. gambianus* ; and the disease in this rat is very similar to the human infection. It shows the emergence of a new serological variant at each relapse, and the appearance of a specific lysin for each variant in turn. If blood is passed to a new animal in the interval between attacks, and an infection is obtained in the new animal, the type of spirochaete is serologically the same as that which appears in the next relapse of the donor animal. That is what might be expected

because some spirochaetes do survive in the intervals, although they are seldom seen in blood films, and those which survive must be new variants. Chart III shows the average titre of the lysin for the attack and first relapse spirochaetes in a series of *C. gambianus.* Several of the rats of this group were re-examined

CHART III.
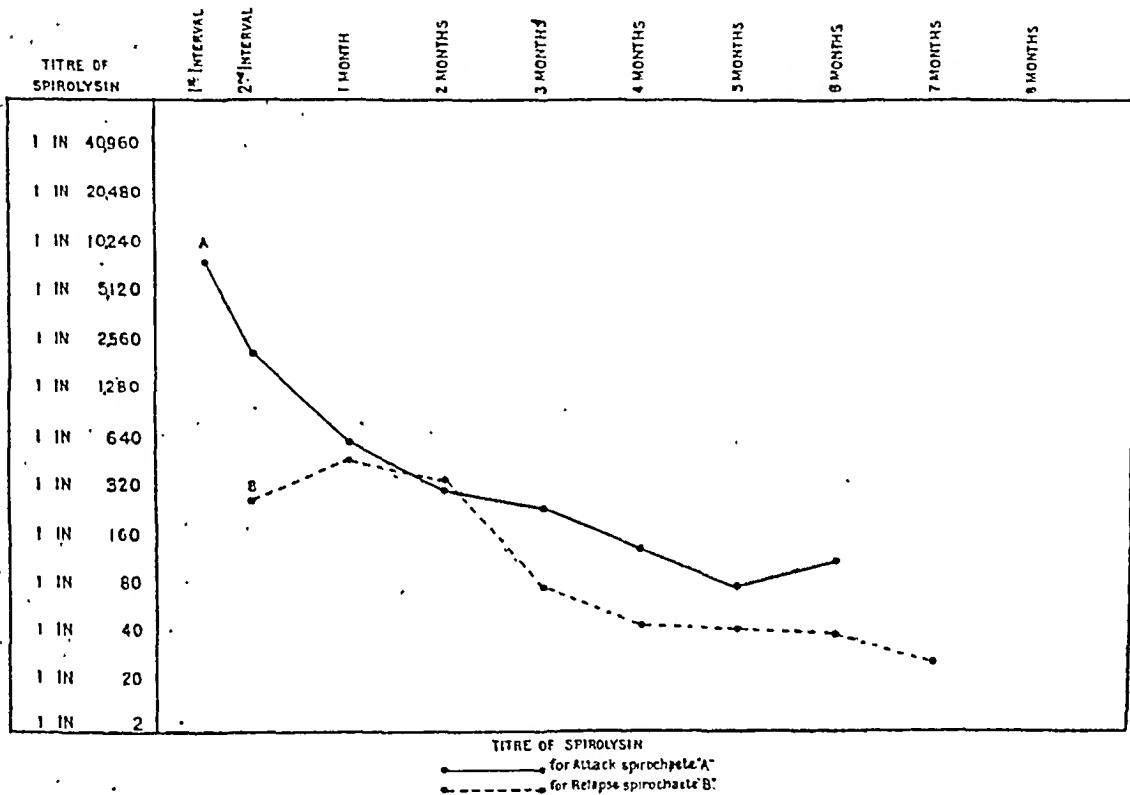
at intervals during the following years and it was found that after 18 months to 2 years the lysin was sufficiently powerful to protect them from infection with the variants which had appeared in their original infections, but that they were susceptible to infection with any other variant.

Chart IV demonstrates the alternation of variants which occurs when relapses are passed on to new animals. That is, that the relapse in the new animal is caused by spirochaetes serologically identical with the attack spirochaetes of the donor animal. This experiment was continued for 5 months before the seventh alternation was achieved, because the strain of relapsing fever available at that time had become weak and failed to relapse in many of the animals.

The special interest in relapsing fever is in the ability of the spirochaete to readapt itself antigenically several times in one host without loss of pathogenicity. However, its possible variations in one host are limited because the

D

CHART IV.
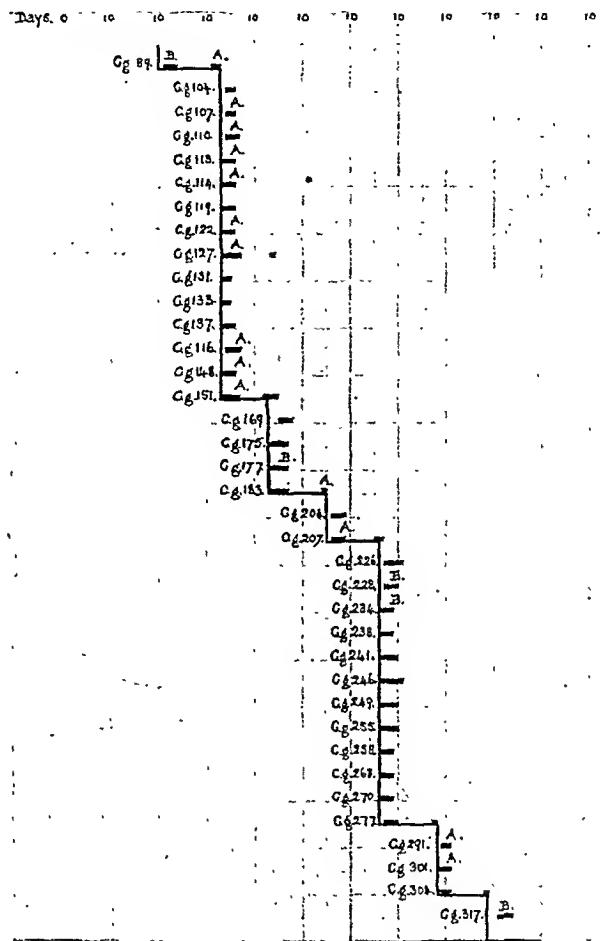
Alternation of types of passage.

A and B = type of spirochaete.
(■) Small black square = 1 day when spirochaetes present in blood.

infection does die out. That might be explained in one of two ways. The body of the host may produce gradually some antibody which is antagonistic to all spirochaetes, but there is no evidence of it so far as the ordinary laboratory technique can detect. On the other hand it is possible that a strain of spirochaete cannot readapt itself more than a few times to the changing serum of one host, and the process may be that of growing old in an ever changing environment.

TRYPANOSOMIASIS.

Trypanosomiasis came into the field of immunological study in the beginning of this century when bacteriological work was well established, and from the beginning it presented difficulties when compared with bacteriological work. No satisfactory method of growing pathogenic trypanosomes *in vitro* was or has been evolved, and there are difficulties in establishing and maintaining satisfactory strains in laboratory animals. The early workers appreciated that immunologically, trypanosomiasis constituted a more complicated problem than bacterial infections appeared to present at that date. FRANKE (1905) found that in monkeys infected with mal de caderas, trypanosomes were present in the circulation at the time when antibodies could be demonstrated, and he assumed that these trypanosomes were insensitive to antibodies. In the Nobel Lecture ERHLICH (1909) referred to this work and added the further observation of his school that many variants of trypanosomes could occur one after the other in one animal. The term " serum-fast " was applied to these relapse trypanosomes and it was assumed that they were variants whose avidity for certain chemical groupings had altered, as was known to occur in drug-fast variants of trypanosomes. RITZ (1916), from his work on experimental trypanosomiasis concluded that the capacity of trypanosomes to vary serologically was inexhaustible, and he used the term " Rezidivstämme " to describe the variants. LEUPOLD (1928) found that considerable variation appears even in the first relapses of animals identically infected with trypanosomes and inadequately treated.

Trypanosomiasis, which is a slow disease in man, may be suitably studied in the rabbit in which the pathogenic strains run a chronic or subacute course. The parasitaemic rapidly fatal strains which develop in mice are less comparable with the disease in man. In the course of the infection in the rabbit trypanosomes are found from time to time in the circulation in small numbers and the skin symptoms and local oedemas progress slowly and certainly to a fatal end. During the last few days trypanosomes may be fairly numerous in the circulation.

Chart V gives the details of an infection in two of a group of rabbits which the writer observed while working in the Liverpool School of Tropical Medicine in 1934. The rabbits were infected with a strain of *T. rhodesiense* and at weekly intervals mice were injected with blood from the rabbits ; at the same time each week some rabbit serum was stored in the ice chest in order that it could be tested for lysins as soon as the mouse infections were positive. It was found

CHART V.

TITRE OF TRYPANOCIDAL ANTIBODY FOR SEROLOGICAL VARIANTS AND DAY ON WHICH EACH WAS ISOLATED IN MICE FROM RABBIT 50·29.

Days on which Serum of Rabbit 50·29 was tested against Variants.		T. rhode-siense Infecting Strain.	Day of Disease.							T. brucei.	T. equinum.
			10	18	24	31	38	45	54		
			Variants.								
			A.	B.	C.	D.	E.	None.	G.		
	Before Infection	0									
	4th day	0									
	10th „	1 : 64	0								
	18th „	1 : 16	1 : 4								
	24th „			1 : 64	0						
	31st „	1 : 64			0						0
	38th „				1 : 64	0	0				
	45th „						1 : 4			0	
	54th „								0		
	80th „		Bayer, 250 mg. per kilo						1 : 16		
	89th „								1 : 4		

that the rabbit serum removed each week contained lysins of low titre for the variant of trypanosome which had been isolated the week before but had no lytic effect on the variant which was present when it was withdrawn. It is likely that a week is not the best interval at which to attempt to isolate new variants, because in each of these two rabbits one attempt to obtain a variant in mice failed completely. There was also evidence, when the animals were very ill, that trypanosomes and some substance which destroyed them *in vitro* were present together in the serum.

Chart VI (p. 188) refers to two other rabbits in which the lysin titre for the infecting variant of trypanosome only was recorded: it illustrates with

CHART V (*continued*). RABBIT 49·29.

	T. rhodesiense Infecting Strain	Day of Disease.							T. brucei.	T. equinum.
		10	18	24	31	38	45	54		
		Variants.								
		A.	B.	C.	None.	E.	F.	G.		
Before Infection	0									
4th day	Tr.									
10th ,,	1:64	0								
18th ,,	1:64	1:64	0							
24th ,,			1:64	0						
38th ,,						0			0	0
45th ,,						1:4				
54th ,,		N.A.B. 50 mg. per kilo					1:16	1:16		
80th ,,	1:16							1:16		

Chart V how weak the lysin is, and shows incidentally that the commonly used drugs do not destroy the antigenic property of the trypanosomes. The technique used to detect lysins was that employed in the Liverpool School to assess the action of trypanocidal drugs, and consists in mixing a known number of living trypanosomes with the weakly immune serum and counting the survivors after some hours. It was found unnecessary to leave the mixtures for 24 hours which is the time chosen for reading the effect of drugs : the lytic antibody destroys the trypanosomes within 6 or 8 hours.

Thus it appears that the course of an infection with a pathogenic trypanosome depends on the capacity of the trypanosome to vary serologically so often in the body of the host that it defeats the possible variations of the host's defence. So the trypanosome strain is relatively immortal in its environment : many years ago LOEB (1913) said that the cancer cell had the same attribute.

CHART VI.

Day of Disease.	Rabbit 815·31	Rabbit 816·31.
	Titre of Lysin for Infecting Trypanosome. Variants not Studied.	
Before Infection	0	0
Infected	T. *rhodesiense* lightly	T. *rhodesiense* heavily
4th day	1 : 8	1 : 12
	{ Treated. with 0·25 grammes trypar- samide per kilo }	
7th „	1 : 24	1 . 32
13th „	1 : 32	1 : 32
20th „	1 : 48	1 : 64
28th „	1 : 32	1 : 64+
34th „	1 : 32	1 : 128+
41st „	1 : 24	1 : 96
48th „	1 : 16	1 : 64
	Apparently cured	Died

GENERAL CONSIDERATIONS.

We have seen therefore that there is evidence that the process of infection in relapsing fever and in trypanosomiasis is maintained by the periodic readaptation of an organism which has invaded the body. The process of resistance in the host, if investigated by ordinary methods for detecting antibodies, is marked by the development of lysins for each variant one after the other. In relapsing fever, which is a blood infection, the appearance of the variants of the spirochaete and the emergence of the corresponding antibody are very well defined and are associated with obvious clinical symptoms. Further, in relapsing fever, the number of spirochaetal variants which can appear in one host is limited. In trypanosomiasis, which is both a blood and a tissue infection, the variants of trypanosome appear one after the other without any great constitutional disturbance, and the number of variants which can appear in one host is limited apparently only by the death of the host.

It is of interest to compare the serological variation of relapsing fever

spirochaetes and trypanosomes with the serological variation of bacteria. The work which has been published on bacterial variation within the last 15 years is immense and it is not within the scope of this paper to do more than to pick out a few of the observations in order to compare what is known of bacterial variation with the variation of spirochaetes and trypanosomes.

In the Harben Lectures, ERHLICH commenting upon FRANKE's description of the Rezidivstämme of trypanosomes remarked that the observation was probably of the greatest importance not only in bacteriology but also in all biology. It is not apparent that the variation of relapsing fever spirochaetes had been investigated by his school at that time, but the study of trypanosome variants was continued, as we have seen, by RITZ in 1916 and LEUPOLD in 1928. As we have seen also the serological variation of relapsing fever spirochaetes was reported by JANCSÓ in 1918 and extensively worked out by CUNNINGHAM and his colleagues later. On the bacteriological side it may be said that the existence of bacterial variants was appreciated by PASTEUR who studied changes in virulence in anthrax and fowl cholera, but it was not until after the War that bacterial variation was appreciated in detail. In 1921 DE KRUIF (1921) demonstrated what he called the G and D types of *Pasteurella lepiseptica*. The D type of the bacillus was highly virulent and produced colonies which were culturally characteristic while the G type was comparatively harmless and showed other appearances on cultivation. About the same time ARKWRIGHT (1921) showed the variants of the coli-typhoid-dysentery group which he called rough and smooth because of the appearance of the colonies on culture medium, the change from the S to the R type being associated with a change in antigenic structure. Since this early work was published it has been shown that very many pathogenic organisms can undergo this type of variation, which is associated with a change in the appearance of growth in various culture media, and with the loss of an antigenic constituent which determines pathogenicity. It has been found that the change from virulent or smooth to non-virulent or rough can be induced artificially in cultures. Adverse conditions of growth, such as changes of temperature and food supply, the presence of weak antiseptics or homologous immune serum, have all been shown to induce it in certain species. From the point of view of this paper the method of growing an organism in its own immune serum is the most interesting of the means by which serological variation has been induced. GRIFFITH (1923) demonstrated it in pneumococcus infection when he found that a pathogenic capsulated pneumococcus grown in homologous antiserum developed into a rough variant which had lost its type specific agglutinability and much of its pathogenicity.

It appears that this variation is a little comparable with the variation which we have been considering in spirochaetes and trypanosomes for in both those infections the variant appears in the presence of homologous antibodies. However, the adaptability of the spirochaetes and trypanosomes is much greater than that of bacteria or cocci, for the variation occurs *in vivo* and all the variants

appear equally pathogenic and survive one after the other in the course of an infection in one host.

So far as present-day knowledge goes we think of disease as caused by a great variety of small forms of life. At one end of the scale of living infective agents are the viruses, and the protozoa are at the other end, the bacteria are in an intermediate position, and any dividing lines which we may please to make are artificial but are an aid to memory. As the result of the serological work of the last half century it has become apparent that the degrees of immunity which the animal body can muster when invaded by these agents are very variable. On the whole, however, it appears that the smallest infective agents, the viruses, induce the most effective and long standing immunity, and they do not seem to be able to readapt themselves serologically in one host. The bacteria are less highly specific and induce less lasting immunity ; they vary serologically, but there is little experimental evidence that serological variation in one host is characteristic of bacterial disease, although the course of some prolonged bacterial infections suggests that variation in the presence of serum antibodies must occur. When we come to spirochaetes, taking relapsing fever as an example, the parasite can vary several times in presence of antibodies, and the appearance of immune bodies in the host is of less significance as evidence of immunity. In trypanosomiasis, an infection with a parasite enormously larger than a virus or bacterium, the infecting agent appears to be more adaptable than the host, and the detection of immune bodies loses the significance which is usually attached to it in the study of disease. To quote ERHLICH again, " the struggle in disease lies between the adaptability of the parasite and that of the host, and the one whose adaptability is the highest remains the victor " (Harben Lecture 2). In trypanosomiasis at least the adaptability of the parasite seems to be on a level with that of the host.

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THE DEVELOPMENT OF ANTISTREPTOLYSINS AND ANTIFIBRINOLYSINS FOLLOWING ACUTE ATTACKS OF RECURRENT TROPICAL LYMPHANGITIS.

BY

P. MORALES-OTERO

AND

A. POMALES-LEBRÓN.

From the School of Tropical Medicine of the University of Puerto Rico, under the auspices of Columbia University.

For many years the etiology of recurrent tropical lymphangitis has been the subject of bitter controversy. There is a group of investigators who believe that the attacks of acute lymphangitis are due to toxins of the filarial worm (*Wuchereria bancrofti*) ; there is a second group which attributes the symptoms of the disease to infection with the well-known pathogen *Streptococcus haemolyticus* ; and there is a third and more conservative group which claims, first, that there may be a lymphangitis purely filarial in origin ; second, that there exists a streptococcal lymphangitis ; and third, that there is a combination of the two conditions in which the worm and the cocci play a combined part.

So far, we must admit that all these hypotheses have been based chiefly on clinical observation, and very little experimental evidence has been reported to prove or to disprove them.

The purpose of this article is to describe the development of antistreptolysins in patients with acute attacks of recurrent tropical lymphangitis, and to describe the resistance to streptococcal fibrinolysis which develops in the blood of these patients following acute recurrent lymphangitis.

ANTISTREPTOLYSINS.

TODD (1932) and COBURN and PAULI (1932) have demonstrated that these antibodies are found in abnormally high concentrations in the sera of patients suffering from rheumatic fever and in individuals recovering from haemolytic streptococcus infections. Furthermore, COBURN has observed that antistreptolysins are not abnormally increased in the sera of subjects with bacterial infection other than haemolytic streptococcus, and he concludes that abnormally high antistreptolysin titre is strong evidence of recent infection by haemolytic streptococci, and that this relationship is apparently specific. While studying the probable relationship of *S. haemolyticus* to recurrent tropical lymphangitis we have made antistreptolysin determinations in a group of persons suffering from this condition, and in a group of normal individuals.

MATERIAL AND METHODS.

The culture medium and the method employed by us in the production of the reduced haemolytic streptococcal haematoxin was that described by HODGE and SWIFT (1933). N.Y.$_5$ scarlet fever strain of *Streptococcus haemolyticus* was used.

For the antistreptolysin determinations we proceeded as follows : Amounts of the reduced filtrate equal to 0·05, 0·10, 0·15, 0·20, 0·25, 0·30, 0·35, 0·40, 0·45, 0·50 c.c. were pipetted into a series of Wassermann test tubes. The volume was made up to 1·5 c.c., in each case with normal saline and 0·5 c.c. of a 5 per cent. suspension of washed fresh rabbit red blood cells in physiological saline added. The contents were mixed and the tubes placed in a water bath at 37° C. for one hour. The smallest amount of filtrate producing complete haemolysis was taken as the minimal haemolytic dose, which, in this case, was 0·2 c.c.

Testing Sera for Antistreptolysin Content.

A preliminary test using serum dilutions of 1 : 25, 1 : 100 and 1 : 500 was made in each case. One c.c. of each dilution was pipetted into three Wassermann test tubes. Two-tenths of the filtrate were added and the volume made up to 1·5 c.c. with normal saline. The contents were mixed and incubated in a water bath at 37° C. for 15 minutes. Five-tenths of 1 c.c. of a 5 per cent. suspension of washed fresh rabbit red blood cells were added, mixed thoroughly, and incubation continued for 45 minutes longer, the tubes being shaken occasionally. At the end of this period the tubes were centrifuged and the highest dilution showing no haemolysis recorded. Assuming this dose to be the 1 : 100 dilution, then a new titration was made of the above dilution ranging from 0·1 c.c. to 1 c.c. in 0·1 c.c. doses, using 0·2 of the filtrate. The smallest amount of serum dilution completely inhibiting haemolysis was then recorded—in this case, 0·2 c.c. Another titration was carried out, ranging from 0·11 to 0·2 in 0·01 c.c.

increments.   The smallest amount of serum dilution that completely inhibited haemolysis was recorded—in this case, 0·15 c.c.   This meant that 0·15 c.c. of the 1 : 100 dilution of the serum tested neutralizes 0·2 c.c. of the lytic filtrate ; in other words, 0·0015 c.c. of the undiluted serum.   In reporting results, the reciprocal of the fraction as recommended by TODD and others is used. We used a given dose of lytic filtrate and our units were not comparable with those of other investigators.   In order to obviate this difficulty we determined the antistreptolysin values of eight of our sera and sent the same samples to Dr. DAVID SEEGAL of New York, who kindly tested them for us.   He reported his results in unit values equivalent to those of TODD, COBURN and others. By calculation, we were able to change our figures accordingly.   In all the tests a serum of known antistreptolysin content was included as control.

TABLE.

ANTISTREPTOLYSIN CONTENT OF THE SERUM IN 69 CASES OF TROPICAL LYMPHANGITIS.

Days after Attack.	Number of Tests.	Result.		
		Maximum.	Minimum.	Average.
0	27	1,111	76	233
1	3	269	138	191
3	4	185	64	124
4	2	238	138	188
6	4	606	25	233
7	2	219	151	185
8	3	333	81	172
9	2	128	104	116
10	3	1,111	41	469
11–90	46	1,041	22	205
730	1	62		
Average of all cases = 198.				

When a series of tests was done at varied intervals in sera from the same patient, each time a new determination was made the previous ones were repeated, to check results and obviate the possibility of error.   Each new batch of streptolysin was standardized with a serum of known antistreptolysin content.

The antistreptolysin content of the sera of patients suffering from attacks of recurrent tropical lymphangitis was determined during the attack, a few days after the attack, and, in some instances, several weeks after the acute attack. The findings are presented in the table above.

The antistreptolysin content varies in different cases and in the same case at different periods. It also varies in the cases that have had only one attack and in those that have had several repeated attacks, as a rule, being higher in the latter. In most cases, the antistreptolysin values of the ·patient's serum are low at the onset of the attack. There is a sharp rise that may occur at any time from a few hours to 7 or 8 days, but usually occurs during the first 24 hours. Then, the titre is more or less sustained for a few weeks, decreasing gradually. From the second month on, the decrease is more rapid, until a second acute attack ensues and the antistreptolysin values rise again. There are several cases that show normal values ; most of them are cases suffering from the first attack, others were investigated from 30 to 60 days after the attack subsided.

According to most authors, the antistreptolysin contents of the blood of normal patients vary from 20 to 100 units. It is really very hard to determine what is a normal case. We tested the serum of twenty apparently normal subjects. Some of them had a previous history of streptococcus infection, especially recurrent sore throat. In this group the antistreptolysin values varied from 18 to 151 units. The average was 64 units.

Fluctuation of Antistreptolysin Content. ·

The fluctuation of antistreptolysin titre in a case of recurrent tropical lymphangitis was studied during the attack, 45 days and 90 days after the attack, and when the next attack issued.

Chart I shows the fluctuation of antistreptolysin titre in a case of recurrent tropical lymphangitis. During the acute attack the antistreptolysin titre was 600 units ; 45 days after the attack the titre was 510 units ; 90 days after the attack, 300 units. The patient left the hospital well but returned with another attack 6 months after being discharged. His antistreptolysin titre then was 750 units. We have made a composite curve with a hundred determinations on different patients at varying intervals. The curve shows that at the onset of symptoms the antistreptolysin values are low (below 100 units) ; there is a sharp
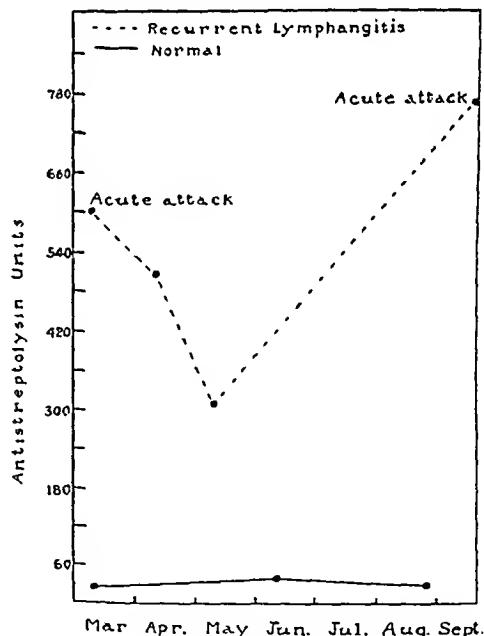
CHART I.

Showing the fluctuation of antistreptolysin titre in a case of recurrent tropical lymphangitis as compared with a normal.

rise usually within the first 48 hours, this value being more or less sustained for several days, and then gradually dropping within 60 days. This is seen in Chart II.
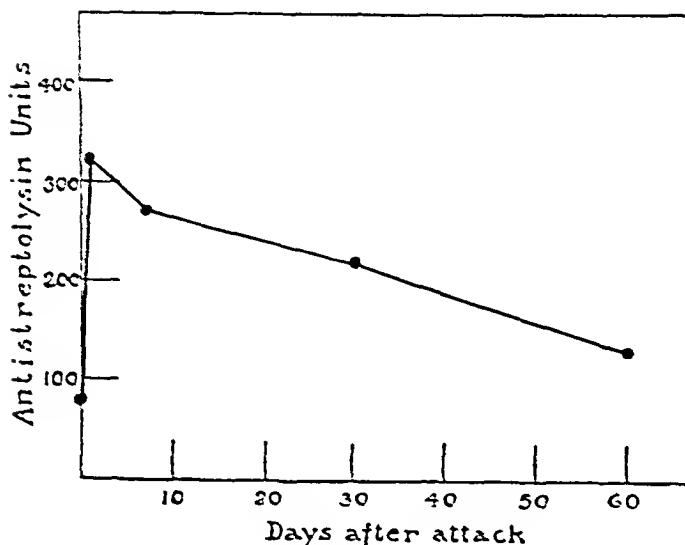
CHART II.

Composite curve of antistreptolysin determinations in cases of recurrent tropical lymphangitis.

There is a definite increase in the antistreptolysin content of the blood of most cases suffering from recurrent tropical lymphangitis if compared with a normal subject. As a rule, there is also a fluctuation in the antistreptolysin content of the blood of these cases, it being lower before the attack and increasing from 1 to 7 days after the onset. The values gradually decrease within 60 days.

ANTIFIBRINOLYTIC DETERMINATIONS IN CASES OF RECURRENT TROPICAL LYMPHANGITIS.

TILLETT and GARNER (1933) have recently shown that broth cultures of haemolytic streptococci of human origin rapidly dissolve human fibrin clot. TILLETT, EDWARDS and GARNER (1934) demonstrated the development of resistance to dissolution in the plasma clot obtained from individuals following acute haemolytic streptococcus infections. They also showed that this antifibrinolytic property is absent in the fibrin clot derived from a group of patients convalescing from other infections. Likewise, the blood from the great majority of healthy adults and from persons suffering from other diseases was found to be susceptible to fibrinolysis. The authors believe that this insusceptibility to dissolution is specifically induced and that " the fibrinolysin of haemolytic streptococci in the body makes a definite response directed against the lytic action of the bacteria."

Forty-one strains of haemolytic streptococci of the beta type isolated in Puerto Rico from cases of various diseases including lymphangitis, osteomyelitis, scarlet fever, septicaemia, etc., were tested as regards their power of dissolving normal human plasma clot. In performing the test the method employed was that recommended by TILLETT, EDWARDS and GARNER, with the exception

that we used tryptic digest broth instead of glucose broth to grow the streptococci. In two cases there was partial dissolution in 24 hours. In the remainder, complete dissolution occurred in 1 to 18 hours. Of the strains isolated from lymphangitis, all but two (L_{21} and L_9) showed a powerful fibrinolysin.

A strain of streptococcus, isolated from the tonsils, which caused dissolution of normal human plasma clot in two-thirds of an hour was tested against the plasma clot obtained from patients suffering from recurrent lymphangitis with a view to estimating any antifibrinolytic action. In all, the blood from 43 cases was tested. Most of the cases showed maximum resistance to dissolution. Some cases showed partial dissolution in 24 hours. In a few cases dissolution was complete in $\frac{1}{3}$ to 22 hours.

The plasma clot derived from cases of recurrent tropical lymphangitis develops a definite resistance to the fibrinolytic activity of haemolytic streptococci. However, in two cases of lymphangitis in which virulent haemolytic streptococci were isolated from a local lesion in the affected limb during the acute attack, fibrinolysis was complete in 30 minutes during the attack ; and in 2 hours, 8 days after the attack.
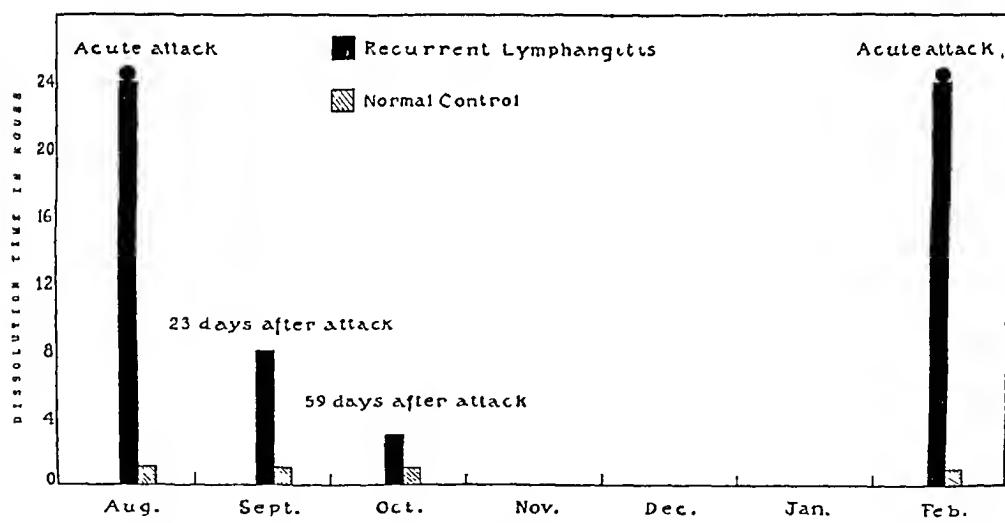
CHART III.

Fluctuation in the antifibrinolysin content of the blood in a case of recurrent tropical lymphangitis.

Repeated determinations at varying intervals were made in several cases of recurrent tropical lymphangitis. In four cases the plasma clot exhibited maximum resistance from the onset of the acute attack to 6 weeks after the attack. In one case, the dissolution time was 14 hours, 2 hours after the onset

of symptoms ; and maximum resistance on the 18th and 59th day after the attack. Another case exhibited maximum resistance when the attack was subsiding and showed complete dissolution in 10 hours, 8 days after the attack ; and in 8 hours, 24 days after the attack. A case came into the hospital during the month of August with an acute attack. His blood plasma showed no dissolution in 24 hours. In the month of September, 23 days after the attack, his plasma showed dissolution in 8 hours ; and during the month of October, 59 days after the attack, his plasma clot dissolved in 3 hours. The patient was discharged from the hospital and came back with another acute attack in the month of February and his plasma clot exhibited complete resistance to dissolution in 24 hours. This is shown in Chart III.

Similar tests carried out with clots from cases of other diseases and normal individuals were made in fifty-two cases. The results were of similar range but were on the average distinctly lower.

In normal controls where repeated determinations were made at short intervals during several months, the dissolution time varied slightly from 30 minutes the lowest, to 2 hours and 30 minutes the highest. This confirms the findings of TILLETT, EDWARDS and GARNER.

In other conditions there were four cases that showed complete resistance to dissolution in 24 hours and it was found that they had a history of previous streptococcus infection. All other cases showed dissolution of the plasma clot at varying intervals, the lowest being 20 minutes, and the highest 15 hours.

SUMMARY.

There is a definite increase in the antistreptolysin content of the blood of most patients suffering from recurrent tropical lymphangitis if compared with normal subjects.

Plasma clot derived from cases of recurrent tropical lymphangitis develops a definite resistance to the fibrinolytic activity of haemolytic streptococci. TODD, COBURN, TILLETT, EDWARDS and GARNER have proved that this occurs only following acute haemolytic streptococcal infections ; therefore, we interpret our findings as evidence that the acute attacks of recurrent tropical lymphangitis are preceded by haemolytic streptococcal infection.

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NOTES ON IMMUNITY IN TRYPANOSOMIASIS.

BY

LL. E. W. BEVAN, M.R.C.V.S.

Beit Research Worker in Trypanosomiasis, Southern Rhodesia.

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In 1909, the writer introduced into Southern Rhodesia the method of treating cattle suffering from trypanosomiasis, with intravenous injections of potassium antimonyl tartrate. Since then the treatment has proved highly successful, not only in Rhodesia, but in other parts of Africa, and has been the means of saving the lives of many thousands of animals.

The method presented one or two practical disadvantages. The operation of intravenous inoculation was somewhat difficult for the layman to perform ; and if the solution escaped into the subcutaneous tissues, it caused swelling, abscesses and sloughs incapacitating working oxen for more or less lengthy periods. For this and other reasons, treatment was often delayed until the animal was too far advanced in the disease; or, if it recovered, convalescence was delayed.

During the 25 years' experience of this treatment in Southern Rhodesia it was frequently observed that animals which had recovered as the result of the treatment could return to the fly area and remain there in good health notwithstanding re-infection. The writer came to the conclusion that a cure to the extent of sterilising the treated animal of trypanosomes was rarely effected and was, indeed, undesirable ; but that it was better to set up a state of tolerance or immunity comparable to that produced in cattle suffering from piroplasmosis when treated with trypan-blue. A " cured " animal would become re-infected and die, whereas an animal, rendered tolerant, resisted re-infection and, indeed, its tolerance appeared to be re-inforced thereby.

On the basis of these observations, therefore, he introduced a method of inoculation against trypanosomiasis which he described in these Transactions (Bevan, 1928) whereby cattle were deliberately infected with known strains of trypanosomes, and were treated at definite intervals indicated by the observation

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of controls, with a view to setting up a state of tolerance. The idea was to get the infection and treatment " over and done with " instead of postponing treatment until the animal was sick from natural infection.

The observation that treated animals on recovery could return to the fly area and remain healthy in spite of re-infection was soon confirmed by veterinarians in other parts of Africa ; and it was pointed out that in some tsetse-infested countries, the cattle naturally acquired a resistance to trypanosomiasis from which they suffered in a latent form until some adverse condition upset the equilibrium and the disease became acute. Thus in Nigeria it was found that apparently healthy cattle were infected with trypanosomiasis which only became apparent after they had been inoculated against rinderpest. Or again, in the Koalib Hills, Nuba Mountain Province, Sudan, where *Glossina morsitans* occurs and is always closely associated with the Nuba inhabitants and their animals, the cattle, sheep and goats are resistant to trypanosomiasis although proved to be carriers of infection. STEWART (1935) states " Trypanosomiasis causes few fatalities in the West African shorthorn cattle of the Gold Coast although it is responsible for greater loss of condition and stunting of size, and though deaths from trypanosomiasis are scanty, the latter disease appears to act as a catalytic agent for other protozoal conditions. . . . This state of high resistance to trypanosomiasis is common to the West African shorthorn throughout West Africa, where that bovine exists in large numbers in French Senegal, Guinea, Ivory Coast, Dahomey, Togo and elsewhere."

The idea of deliberately setting up a tolerance as suggested by the writer was assailed on the grounds that immunity conferred against one strain of trypanosome might break down when the animal was exposed to infection with another strain. This objection was subsequently discounted by those who raised it when it was found that re-infection with a different strain of the same species produced in a properly nourished animal merely a transient anaemia. In any case, by inoculating animals with the virus of the area to which they were to be exposed, this difficulty could be overcome.

In practice, however, it was found that breakdown of tolerance did occur, but from other causes. It occurred when the animals became infected with a species of trypanosome against which they had not been rendered tolerant, as for example, when cattle inoculated against *Trypanosoma congolense* became infected with *T. vivax*. Starvation, over-work, mineral and vitamin deficiencies intercurrent infections such as piroplasmosis, anaplasmosis, verminosis and rinderpest were found to cause breakdown of resistance. To render the method of protective inoculation entirely effective, it would be necessary to revolutionize the methods of animal husbandry practised in fly areas and to protect inoculated animals from all the other diseases they might encounter—an almost impossible proposition.

Another objection to any method based upon tolerance was that the animals became carriers of infection and a possible source of danger to uninoculated

animals. Their introduction into other tsetse-infested areas might infect " clean " fly. The danger was not as great as it was represented to be, because in such areas the game already provide the tsetse with trypanosomes. However, it became obvious that an alternative process based upon a firm " sterile immunity " rather than an immunity tolerance, was desirable, and it was decided to endeavour to devise such a method. The problem had to be approached *de novo*, and it was thought wise to enquire how resistance was brought about in nature, and whether such a condition as complete immunity rather than tolerance was ever effected.

Although many of the early hunters and explorers of Africa during the last century encountered and described the tsetse fly and were well aware that its bite was deadly to domestic animals, they did not attempt to explain why the wild animals which were constantly bitten by it apparently resisted infection and could live in tsetse-infested areas with impunity. The resistance of young domestic animals to trypanosomiasis, however, is frequently referred to in the old records. Thus LIVINGSTONE (1857) states that the fly is harmless to calves " so long as they continue to suck the cows," although he says that dogs perish if fed on milk. BAINES (1877) many years later said very much the same thing, namely, that by driving calves into the fly area during the day and bringing them out to be suckled night and morning they might be rendered secure during the rest of their lives. SELOUS (1881) refers to the resistance of young domestic animals, and BRADSHAW (1881) records that " if dogs are taken into the fly district by the bushman, when pups, and are allowed to suckle the mother, and at the same time given as much " fly " to eat as can be captured for them, the mother will die but the pups will live and grow up." Dr. A. MACKENZIE, who has a unique knowledge of the natives in the Southern Rhodesia fly areas, informs the writer that in some parts they own large flocks of sheep which originate from pregnant ewes purchased from fly-free areas. Although most of the mothers die, a large proportion of their progeny survive and provide the nucleus of the flock. SCHILLING (1933) in an article " Immunisierung gegen Trypanosomen-Krankheiten " states that in Central Africa, where the diseases transmitted by tsetse are met with, numerous solipeds and ruminants live in the savage state and resist trypanosomiasis, owing to an immunity or tolerance acquired during their young days and re-inforced all their lives by the bite of tsetse flies. He advocates the vaccination of young animals with a view to giving them a labile infection during their early days and reinforcing their resistance by the bite of infective tsetse flies. Dr. SCHILLING has patented his process of inoculation and from the specification it would appear that he deliberately infects the young animal and at the appropriate time " shuts off " the reaction with antimony, very much in the same manner as that suggested by BEVAN (1928) for the protective inoculation of cattle.

It is probable that in the fly-infested areas the common method by which the game become infected is by the bite of the tsetse fly. But the chances of

infection are unequal. For example, the density of the fly and the proportion of infective flies vary. Some animals are migratory, others more or less stationary ; some are more conspicuous than others, and some are diurnal, others nocturnal, in their habits. It is also known that infection may be transmitted mechanically not only by the tsetse fly but by other blood sucking insects, but in all probability the tsetse is, in most outbreaks, the initiating factor. There are, of course, other methods whereby the game may become infected. BASSETT-SMITH (1919) showed that trypanosomes occurred in the organs of the foetuses of rats infected with *T. rhodesiense*, and in 1921 noted that young guineapigs born of a parent infected with *T. gambiense* showed trypanosomes in the blood about a month after birth. Recently LYNDHURST DUKE has recorded that five guineapigs with *T. rhodesiense* in their peripheral blood gave birth to young which were found to be infected. BEVAN (1911) sought to determine whether the lambs of infected mothers could become infected *in utero*, but no lamb born of an infected mother was ever found to be infected. He wrote, " Experiments have been conducted which have proved that the tissues of a foetus of a highly infected mother are not infective ; also that the milk of an infected ewe does not convey infection to the lamb feeding upon it, or to animals artificially inoculated with it. Lambs feeding upon such milk derive no immunity therefrom."

In the endeavour to elaborate a method of protective inoculation resulting in a sterile immunity as distinct from tolerance, experiments were carried out with rats and mice, using a very virulent strain of *T. rhodesiense* as the infecting agent. In adult rats and mice the average period of inoculation was 6 days, and the average duration of the disease was 21 days. It was realised that rats and mice were not ideal subjects for the work, but, *faute de mieux*, they had to be employed. Rabbits were unsuitable because when disturbed they destroyed their young. Dogs were difficult to obtain, and no accommodation was available for pigs. Guineapigs did not yield sufficiently large litters. Horses, cattle and sheep were open to the same objection and were too expensive. Considerable difficulty was experienced with rats which also devoured their young if they were disturbed or the young were handled for inoculation. This, however, was over-come to some extent by removing the mother before touching the young, and painting her nose with aniseed or eucalyptus before returning her. It usually happened, also, that if the mother was infected she would destroy or devour her young when she found herself unable to nourish them. Space does not permit a description in detail of the many experiments which ended in failure, but some of the positive results may prove of interest.

The foetuses of infected pregnant rats which had been destroyed by chloroform, were removed, carefully flamed and afterwards decapitated, their blood being collected in citrate solution. They were then opened and eviscerated. The heart, lungs, liver, kidneys and spleen were collected and ground with sterile sand in citrate solution. The mixture with the citrated

blood was filtered through lint and 1 c.c. was injected into healthy rats, none of which ever became infected. A similar experiment was performed with three newly born young of an infected rat. The tissue mixture inoculated into two rats failed to infect them. A third young rat of the same litter had a damaged urachus and what appeared to be a subcutaneous clot. This animal was treated in the same manner and a rat inoculated with the mixture became infected after an unusually long period of incubation.

The young of infected rats and mice were never found to be infected at birth or after, even when cohabitating with the infected mother or taking her milk. They were, however, susceptible to syringe infection. When the mothers were treated with Bayer 205 the course of the disease in the young was not arrested.

Young mice or rats of infected or healthy mothers could be infected by syringe inoculation. If well nourished the period of incubation was longer than in adult rats and the duration of the disease might be prolonged for as long as 6 months. They might eventually recover, *but if they recovered they were again susceptible to re-infection.* If, however, they were re-inoculated during their first infection and thereafter, the infection was prolonged and a state of tolerance was acquired. The process, however, was largely dependent upon proper nutrition. The weaklings were gradually crowded out by the strong and died. Later, as the members of the litter grew, the males worried the females and some of them died. Eventually the males fought and killed each other. At the long last only one or two of the litter survived and unless re-inoculated they lost their infection. It was found that survival was dependent upon adequate nourishment and it was always necessary to reduce a large litter to three or four. When the mother died and the young had to be weaned, the greatest care had to be exercised in providing them with a suitable diet. Any deficiency resulted in the death of the young from trypanosomiasis. One mouse which gave birth to five young ones devoured four of them, the one which was spared was inoculated and became infected, it grew rapidly and although still infected became larger than its mother, and eventually it fought and killed her. About a month later " Little Benjamin " was also found dead—possibly from remorse but certainly not from trypanosomiasis.

It may also be mentioned that no mother rat ever became infected from devouring her infected young or by cohabitating with them ; and control non-infected young did not contract the disease from the infected members of the litter. Mice were fed on bread soaked in citrated infected blood and neither became infected nor immune. Three cannibal rats were frequently given the carcases of their dead infected comrades which they devoured almost completely, but did not become infected. One male mouse cohabitated for 3 months with infected females and did not become infected. The females did not live long enough to bear young, but coitus undoubtedly occurred.

It is fully appreciated that these results are contrary to those of other workers, and it may be that the technique adopted was at fault, or that rats and mice do not yield true results. With regard to the absence of infection *in utero* it may be that the placental arrangement in the different species is not equally effective, although the filtering action of the placental barrier would not, as a rule, permit the passage of a body of such dimensions as a trypanosome.

It would, however, appear from these experiments that young rats and mice are susceptible to infection with trypanosomiasis, but possess some degree of resistance which, provided they are well nourished and not unduly disturbed, enables them to recover from an infection of sufficient virulence to kill the weaklings or adult animals. If complete (sterile) recovery takes place they again become susceptible and on re-infection may die. But if re-infected during the course of the early infection and from time to time thereafter, a state of tolerance is acquired. This may be what happens in the case of the wild animals in tsetse-infested areas. It is comparable to that which happens with calves subjected to natural infection with piroplasmosis conveyed by ticks. Calves born on tick-infested veld contract the disease in a mild form and if frequently reinfected become tolerant, but if removed to areas free from ticks lose their infection and their immunity. They again become susceptible and may even die of infection. This is probably what happens to children in hyperendemic malaria districts.

The necessity for re-infection in the creation of tolerance has also a bearing upon the peculiar incidence of human sleeping sickness in Southern Rhodesia. From time to time cases have been detected and as recorded in the *Journal of Comparative Pathology and Therapeutics* (1935), lviii (June), a case was recently discovered at a village called Gowe which was known as a place at which Europeans and natives—even from the neighbouring tsetse-infested country—have become infected with a deadly form of trypanosomiasis. The latest subject was an old native *Kahondera* who was found to be suffering from the disease in a latent form, although his blood was highly infective to laboratory animals. It was ascertained that he had been born on the Nyopakwe River quite close to Gowe, and had lived there all his life. On studying the maps compiled by JACK (1933) from the records of the old hunters, explorers and officials, it was found that Gowe was one of the few, small, well-defined areas in which the tsetse fly persisted after the rinderpest epizootic of 1896. It is only in such areas that repeated re-infection by tsetse during the past 60 years could have occurred. There are about eight such " residual foci " of tsetse defined on the map and it is significant that in 1913 similar cases were found in the Manzituba Vlei, which is another of them. Although in many parts of the 20,000 square miles of Southern Rhodesia in which *G. morsitans* occurs, *T. brucei* has been met with in domestic animals and game, sleeping sickness in man has only been found to be endemic in these two areas. It would, therefore, appear that the specific trypanosome of man is transmitted from man rather

than from game. It is suggested that by evacuating the natives from the endemic areas to fly-free country, sleeping sickness will disappear from Southern Rhodesia, unless introduced from neighbouring territories.

The experiments have a practical application in connection with the inoculation process in that it would appear that the tolerance of the inoculated cattle must be maintained by frequent re-infection. In districts where tsetse are scarce or only occasional visitors, the tolerance of inoculated cattle may die out and animals subsequently re-infected may die. It should be reinforced by repeated re-inoculations at appropriate intervals, the inoculations being performed before the tolerance has completely died out.In districts where tsetse are numerous and constantly present, artificial re-infection may not be necessary. In all cases the general health of the animal must be maintained by suitable nourishment and reasonable care.

The maintenance of tolerance being dependent upon the persistence of infection, entails the creation of carriers or reservoirs of infection—a potential danger to uninoculated animals. Therefore, a firm sterile immunity comparable to that set up against certain bacterial and virus diseases would be preferable. If some simple and safe method of setting up in man and animals a complete resistance to trypanosomiasis could be devised—and in the light of recent progress it does not appear to be beyond the bounds of possibility—the tsetse fly would be rendered innocuous. Large sums of money are being spent in the attempt to eradicate the tsetse, but before complete success in this direction can be claimed, it is necessary to " swat " the last fly—an almost impossible proposition under the conditions obtaining in Central Africa. The attempt to protect animals against the trypanosome which is the actual cause of the trouble, would appear to offer a greater prospect of success.

SUMMARY.

1. The treatment of cattle suffering from trypanosomiasis with intravenous injections of potassium antimonyl tartrate was introduced into Southern Rhodesia in 1909 and has since been adopted with considerable success.

2. In the course of 25 years' experience, it has frequently been observed that treated recovered animals may return to the tsetse-infested areas and remain there apparently in good health in spite of re-infection. This observation has been confirmed by veterinarians in other parts of Africa.

3. Treated animals either are sterilised of the parasite and again become susceptible to re-infection, or are not completely sterilised and become tolerant.

4. A method of inoculation and treatment based upon these observations was introduced in 1928. It consisted of the deliberate infection of cattle with known strains of trypanosomes—preferably the strains of the district in which the animals were to be exposed—and of treating them at the appropriate time.

5. In this manner they were rendered tolerant or immune, but in practice it was found that their tolerance broke down when the animals were subjected

to adverse conditions. To obviate breakdown would involve a revolution of present-day conditions of animal husbandry in tsetse-infested areas.

6. Tolerant animals become reservoirs of infection ; therefore a complete sterile immunity, rather than a tolerance, would be preferable.

7. In nature the resistance of the wild animals in tsetse-infested areas is, in most cases, a tolerance. A similar condition is sometimes met with in indigenous domestic animals.

8. Old hunters and explorers have attributed this resistance to the infection of young animals during their early days.

9. Recent experiments with rats and mice indicate that young animals are susceptible to infection ; but if well nourished and not unduly disturbed, they may survive an infection sufficiently virulent to kill weaklings or adults. If they recover, they again become susceptible ; but if re-infected during the course of the original infection, they become tolerant.

10. These observations indicate how tolerance may be acquired in nature and suggest that it is maintained by repeated re-infection.

11. They explain the peculiar incidence of latent cases of sleeping sickness in Southern Rhodesia and suggest preventive measures.

12. They also indicate that the tolerance of inoculated cattle should be maintained by repeated syringe or fly infection.

13. The integral properties of the young which render it partly resistant have not been determined, but appear to be worthy of further research.

14. A method of setting up a complete and lasting sterile immunity in man and animals against trypanosomiasis comparable to that commonly set up against bacterial and virus diseases appears to offer a solution to the tsetse fly problem.

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A SECOND NOTE ON A HIGH RATE OF INFECTION OF THE SALIVARY GLANDS OF *GLOSSINA MORSITANS* AFTER FEEDING ON A REEDBUCK INFECTED WITH *TRYPANOSOMA RHODESIENSE*.

BY

J. F. CORSON.

From the Research Laboratory, Tinde, Tanganyika Territory.

Two experiments were recorded last year (CORSON, 1935) in which the rates of infection of the salivary glands of *Glossina morsitans*, which had fed on a reedbuck, infected with *Trypanosoma rhodesiense*, were 60 and 33·3 per cent. respectively. The rate in a control experiment with a monkey was only 1·1 per cent., although the infection in the monkey was acute, showed numerous trypanosomes of all the usual forms and terminated fatally on the 36th day after infection.

The description of the following control experiment which was made a little later has not hitherto been published. Two sheep were infected by flies

from the above-mentioned reedbuck and then boxes of laboratory-bred flies were fed on them for 9 days, after which the flies were fed on " clean " sheep. On the 25th, 26th and 27th days after their first feed on the infected sheep, the flies, which were all *G. morsitans*, were dissected. One batch of 110 surviving flies had three, or 2·7 per cent., with infected salivary glands, while the other batch of fifty surviving flies had none. The reedbuck used in this experiment was darker and greyer than the kind usually seen in the Tinde district, of which there are several examples on the laboratory premises. The grey kind is distinguished by the local natives by the name " mongee " from the usual kind which they call " njaa."

In December, 1934, not long after the " mongee " experiment, two boxes of *G. morsitans* were fed similarly on an ordinary reedbuck (" njaa ") which had been infected with the same strain of *T. rhodesiense* by a single *G. morsitans* on July 25th, 1934. Trypanosomes were present in the reedbuck's blood but were few in number. In January, 1935, the remaining flies were fed singly on white rats and two infective flies were isolated, two rats having become infected. The flies, thirty-two in number, were then killed and dissected and two, or 6 per cent., were found with infected salivary glands. This observation was made by Dr. CALWELL and Mr. SMITH during my absence on leave.

On December 24th, 1935, after my return to Tinde, another specimen of the grey kind of reedbuck (" mongee ") was brought to the laboratory. It was very young and was fed with cow's milk although it could eat grass and leaves, and its health remained fairly good. Another strain of *T. rhodesiense* had been transmitted by *G. morsitans* from a patient to a sheep, during the period October to December, 1934, and had been maintained in sheep during 1935 by fly-passages. It was decided to infect this young reedbuck and then to feed *G. morsitans* on it to see if a high rate of infection of the salivary glands of the flies would again take place.

Accordingly on the 25th February, 1936, an isolated infective fly of the seventh fly-passage, fly BE46, which infected man soon afterwards, fed on the reedbuck. On 28th February the blood of the reedbuck showed no trypanosomes in stained thick films, but on 2nd March, when it was again examined, it showed numerous polymorphic trypanosomes. On the 4th March, four boxes of laboratory-bred *G. morsitans*, labelled K 52, 53, 54 and 55, each containing about thirty flies, were put to feed on the reedbuck, whose blood, on this day, showed numerous trypanosomes. Over half of the flies fed. They were again put to feed on the reedbuck on the 6th March, the trypanosomes in the blood being much fewer. Three white rats were inoculated with the reedbuck blood on this day and all became infected. On the 8th March the flies were put to feed for the third time on the reedbuck, and two new boxes K 56 and 57, were added. On the 10th March all the flies were put to feed on rabbits, and were fed on them daily until the end of March. On the 1st April, 28 days after their first feed on the reedbuck the surviving flies, sixty in number, of boxes

K 52, 53, 54 and 55 were dissected by a modification of Lloyd and Johnson's method which has been described (CORSON, 1933). They were first put singly into test tubes and chloroformed lightly just before dissection, the motility of the trypanosomes in the salivary glands not being affected. Of the sixty flies, twenty-six, or 43·3 per cent. showed infected salivary glands. On the 2nd April the flies of boxes K 56 and 57 were also dissected. Box 56 had twenty-five

TABLE I.

Transmission. (S=sheep.)	Dates of Feeding.	Remaining Flies Dissected.	Infected Salivary Glands.	Per Cent.	Flies Fed on Rats.	Rats Infected.	Per Cent.
Man to sheep	21.10.34				172	4	2·3
S. 131 to S.152	27.10.35 to 9.11.35	69	5	7·2			
S.152 to monkeys	25.11.35 to 10.12.35				33	2	6
S.152 to S.141	29.12.35 to 15. 1.36				54	2	3·7
S.149 to S.150	13. 1.36 to 24. 1.36				160	7	4·3
S.150 to S.160	25. 2.36 and 2. 3.36 to 16. 3.36	63	2	3·2			
S.141 to S.150	14. 2.36 to 4. 3.36				60	3	5

remaining flies and sixteen, or 64 per cent., had infected salivary glands. Box 57 contained only six flies of which one had infected salivary glands. Altogether, forty-three out of ninety-one flies, or 47 per cent., had infected salivary glands. The dissections and microscopical examinations of the glands which were successfully removed from every fly were carried out by myself. Some other figures relating to this strain of *T. rhodesiense*, were available for comparison, as some dissections were made of flies transmitting the infection from sheep to sheep, and infective flies were isolated by feeding on rats. They are shown in Table I.

Some figures relating to the transmission of *T. rhodesiense* from antelopes by *G. palpalis*, compiled from tables published by DUKE (1935a) may be mentioned. Although DUKE does not give the numbers of flies which lived to the 25th day after the first feed, the rates of infection of the salivary glands are so low that, if only one-fifth of his flies survived to the 25th day, the highest rate, that from Reedbuck II, which seems to have become infected accidentally, would be only 8·5 per cent. The figures seem therefore to be comparable to some extent.ᐧ They are shown in tabular form below.

TABLE II.

Infected Animal.	Number of Flies Fed.	Infected Glands on 25th Day.	Percentage of Total Flies Fed.
Reedbuck I	472	4	0·85
„ II	363	6	1·7
„ III	440	5	1·1
„ IV	520	1	0·2
Bushbuck I	488	2	0·41
	516	3	0·6
	591	4	0·7
„ III	616	5	0·8
Oribi I	403	1	0·25
„ II	430	0	0

DISCUSSION.

In the previous paper (CORSON, 1935) it was suggested that the blood of the " mongee " reedbuck of the experiment (not the blood of other reedbucks not in the experiment), may have been especially suitable for development of the trypanosomes in the bodies of *G. morsitans*. ROBERTSON's suggestion that the conditions in antelopes favour the selection of resistant trypanosomes, which are also probably well fitted to develop in tsetse flies, was also mentioned. Some comments on this suggestion of mine were made (DUKE, 1935b) including the remark that ROBERTSON's views on the existence of an endogenous cycle of *T. gambiense* in monkeys, had been overlooked. This was not the case, but ROBERTSON's suggestion mentioned above was regarded as a more likely alternative explanation to my own suggestion. In infections of animals with *T. rhodesiense*, where there is often little indication of a relapsing character, it is conceivable that failure to infect many flies, when the flies were only fed once

or twice on the infected animal, might be due to there being an insufficient number of the short forms of trypanosomes in a healthy condition at the times of feeding. So many experiments have been made, however, in which daily feeding has been carried on for long periods on animals with an acute *T. rhodesiense* infection in which numerous trypanosomes of all the well-known forms were present in the blood, that the theory of an endogenous cycle is insufficient to explain the low rates of infection of the salivary glands which have resulted.

The following short experiment was made. Three boxes of laboratory-bred *G. morsitans* were fed on Monkey 34 (which was infected with *T. rhodesiense*) during the period the 10th to the 22nd January, 1936, and the flies were then fed daily on three guineapigs, one box to each, during the period the 25th January to the 24th February. The guineapigs did not become infected and the thirty-five remaining flies were dissected and no infected salivary glands were found. The blood of the monkey was examined by me in stained thin films daily from the 10th to the 17th January and the trypanosomes were always numerous, most of them being short and intermediate forms, though no posterior-nuclear forms were seen. The strain of trypanosomes, *T. rhodesiense*, Kahama, was that used in the " mongee " experiment described above and in the transmissions through sheep shown in the table. The chief interest, however, of this experiment with the " mongee " reedbuck is the fact that the only two reedbucks of this kind which have been available for experiment have given such high rates of infection of the salivary glands of *G. morsitans*. It appears as if the rate of infection of the flies is determined to some extent by the species of animal from which they derive their infection. It seems to be desirable that surveys of wild animals in sleeping sickness areas should be made ; as well as experiments with single animals of different species, and with various combinations of those animals most frequently met with in association with one another and with tsetse flies in sleeping sickness areas. To use *G. morsitans* for *T. rhodesiense* and *G. palpalis* for *T. gambiense* would seem to be the best procedure.

SUMMARY.

Another instance of a high rate of infection of the salivary glands of *G. morsitans*, after feeding on an infected reedbuck, is recorded ; and it is suggested that the species of animal may be a determining factor in the rate of infection of the flies. Some other figures are given for comparison. ROBERTSON's views on an endogenous cycle of *T. gambiense* in monkeys are referred to and the opinion is expressed that they are not applicable to infections with *T. rhodesiense*. It is suggested that surveys of animals in sleeping sickness areas are needed and also further experiments with single animals and with groups of animals.

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THE INVESTIGATION OF AN OUTBREAK OF SLEEPING SICKNESS IN NORTHERN RHODESIA.

BY

HUMPHREY GILKES, M.D.*

Colonial Medical Service (Northern Rhodesia).

Trypanosomiasis in Northern Rhodesia is carried by both *Glossina morsitans* and *G. palpalis*. The latter is confined to a few well defined areas round the shore of Lake Tanganyika and the Luapula River. *G. morsitans* on the other hand infests nearly half of the country and by reason of the virulence and high fatality rate of the disease which it carries it presents a much greater problem. The epidemiology and symptoms of sleeping sickness carried by *G. morsitans* as met with in Northern Rhodesia appear to differ in some respects from the rather scanty descriptions in the text books, and this paper is an attempt to describe the problems which are encountered when an outbreak occurs.

GEOGRAPHY.

Northern Rhodesia is divided approximately into two halves by the railway line which runs northward up the centre of Africa from Cape Town to the Congo. The area concerned in the present outbreak lies to the west of this central railway and is conveniently bounded on the north, the west and the south by a hook which the Kafue River makes before it flows eastward to the Zambesi. Mumbwa, the Government station which administers the district where the outbreak occurred, lies at the centre of the hook. It is situated rather in the position which the town of Ypres occupied to the salient made by the front line during the Great War, the distances being of course much greater. It is a little over 100 miles from Mumbwa to the tip of the salient. The northern part of the hook is a game reserve, though scattered groups of villages were still situated there at the time of the investigation. The southern half is a native reserve, peopled by the offshoots of a considerable number of tribes. A proposed road to the west was sited to run between the game and native reserves, and it was partly in connection with this road that the investigation was undertaken.
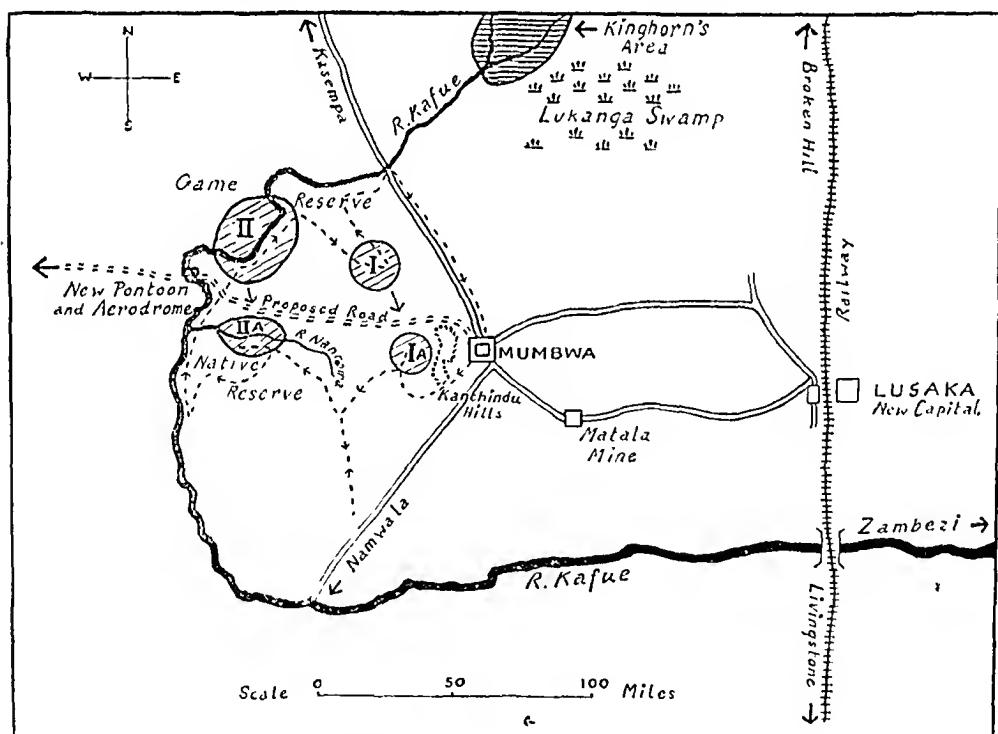
HOOK OF THE KAFUE RIVER, NORTHERN RHODESIA.

Dotted line = course of journey starting from Mumbwa with deviations to cover all villages.

Areas I and II = groups of villages which it is proposed to move to Areas IA and IIA.

The diagrammatic map on page 214 shows more clearly this circumscribed area which is known locally as the Hook of the Kafue. There are also certain areas in the game reserve which are labelled Area I, II, etc., and which will be referred to later in detail. Area I is a group of villages in the eastern part of the game reserve which appeared to be a definite focus of infection. It will be seen later that it is proposed to move these villages to Area IA. Area II is a scattered group of villages in the western part of the game reserve, some of them on the west side of the Kafue and therefore not strictly belonging to the Hook. These villages were already on the move from the game reserve into the native reserve. Their inhabitants were living in temporary crudely built huts while they travelled continually between their old and new homes and attended to their old gardens. They were repeatedly exposed to the bites of tsetse flies and they moved through a district full of game. This area was considered to be another probable source of infection.

It will be noted that the villages of both Area I and Area II are to be moved to new sites during 1936. This will leave the game reserve free of villages. This evacuation of the game reserve is of great importance from the medical point of view.

HISTORY.

Reports of suspected sporadic cases had been received from this district for more than 10 years but it was always supposed that these natives had contracted the disease in a known sleeping sickness area some 100 miles to the north-east. This is known locally as Kinghorn's Area. It is probable that cases have long occurred unrecognised beyond the boundaries of this area, since it is unlikely that in a part of the country where game and fly are so numerous sleeping sickness has been confined to an oval drawn on a map. In 1929 suspicious but undiagnosed cases were recorded in the Hook of the Kafue and in 1934 five suspects were reported, one of whom was diagnosed by blood examination.

The year 1935 saw a great increase in cases. It was difficult to separate the true from the false since both natives and unqualified Europeans sent word of reputed cases and deaths throughout the year. In Northern Rhodesia trypanosomiasis carried by *G. morsitans* resembles in its early stages subtertian malaria, and in its late stages it may be confused by unqualified people with almost any disease showing symptoms of coma or wasting. The presence of enlarged glands at the back of the neck is moreover no true criterion. These enlarged glands are also present in filariasis, a very common disease in the Hook of the Kafue and in various septic conditions of the head and neck. They are commonly absent in early acute sleeping sickness cases.

It is true that natives in sleeping sickness areas acquire facility in recognising cases but the number which they report is always greatly in excess of the true figure. The only reliable guide must be the microscope. Therefore, with the exception of the small group of villages in Area I in which twenty-five suspected

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deaths occurred in the year, and in which the natives had obviously learned from experience of many cases, reports of cases were not considered reliable.

During 1935 there were twenty-nine microscopically diagnosed cases from the Hook of the Kafue. It was felt that it was not profitable to guess at the number of unrecognised cases, and useless to give figures other than this. Nine of these were found during the investigation and the remaining twenty were microscopically diagnosed from blood and gland fluid slides sent in during the year. The figure nine probably represents quite fairly the average number of cases in the district at any given time. It is a reasonable figure for an endemic area of this size in Northern Rhodesia for it accords with the experience of the last 25 years in other parts of the country. The investigation was undertaken in order to settle the following points which had been raised by the District Administration :—

1. The endemicity or epidemicity of the disease.

2. The liability of travellers (European and native) to and from the west, to contract the disease ; and, if necessary, the control of the various routes by which natives from these remote districts come to the railway line to seek work.

3. The desirability of opening the game reserve in the Hook of the Kafue as a tourist centre.

4. The question of moving the natives out of the native reserve if sleeping sickness were severe there.

5. The consideration of the best places to which the groups of villages still in the game reserve should be moved.

6. A site for a new native dispensary.

7. The bearing of sleeping sickness on the native cotton industry which is being started in the native reserve.

8. The advisability of building through the area the contemplated road to the west.

9. A site for an emergency landing ground for aircraft and its best situation from the point of view of sleeping sickness.

Details of the Investigation.

Starting from Mumbwa, the central point of the salient made by the Hook of the Kafue, the course of the journey followed roughly the dotted line shown on the map with wide deviations daily to cover all the villages. Eighty-one villages in all were examined. The population of these, as is usual nowadays in Northern Rhodesia, consisted chiefly of old men, women and children. The young men of the present time who correspond to the warriors of the past find their outlet for adventure by working in the towns, on the railway line or at the copper mines. The country covered by the tour was the whole of the sleeping sickness area of the Mumbwa district.

Native Tribes.

There is a wide variety of tribes collected in this rather sparsely populated area. They range from the Baila, whose scanty clothing renders them especially susceptible to the bites of the tsetse fly, to the more progressive Bakaonde who live in better huts and who have advanced as far as building deep pit latrines in many of their villages. They inhabit the middle of the district and also Area I. The Ambwera, who belong rightly to the country further west and speak a dialect reminiscent of Sekololo and Sesuto, are on the move from Area II into the native reserve to the south. Basala, Baluba, Balenje and Walamba are all represented, and a number of travellers from Barotseland and the extreme west were encountered at various villages. These are passing through continually on their way to become industrialized. They wear little clothing and a number of the cases in 1935 occurred amongst them. They are employed on their way by the local natives as expert thatchers and builders. They follow certain regular routes and earn their food and shelter for the journey in this manner. It will be seen that one of these routes passes through and another near to the highly suspected Area I.

Vegetation.

A large part of the high ground of the Hook of the Kafue is covered by the forest known in East Africa as " miombo," separated by broad wide clearings destitute of trees. A small stream usually flows down the centre of these and in the wet season the clearings are water-logged. These large grass spaces at the time of the investigation (December, 1935) were almost dry. They led by gentle slopes down to the thickets round the rivers which flow into the Kafue River. At the extreme western part of the Hook this country changes to one with the smaller trees of " orchard bush."

Game.

Large herds of wildebeeste, hartebeeste, oribi and impala can be seen in the game reserve but no elephant are found. The association of trypanosomiasis carried by *G. morsitans* with herds of buffalo is well known, and it is borne out again by the large herds which roam through this game reserve.

Tsetse Fly.

The daily catch of tsetse fly which was made regularly at various places and times showed only *G. morsitans*. The whole of the Hook of the Kafue appears to be infested with them, the limit of the fly eastwards being a small salient round Mumbwa at a distance of about 25 miles from the station. In December when the tour was undertaken the rains had already come, the bush and grass had begun to grow and the fly were more widely dispersed throughout the country than in the hot dry months.

DISTRIBUTION OF CASES OCCURRING IN 1935.

The most accurate view of the situation in this area is obtained when the twenty-nine diagnosed cases are considered in detail as follows :

A.   Six Cases from Matala Mine.

This is a gold mine 27 miles east of Mumbwa in a fly-free district.   The compound manager, a layman, had had experience in the Belgian Congo of trypanosomiasis carried by *G. palpalis*.   The mine owns a small hospital in charge of a trained nurse and twelve reputed cases passed through their hands during the year.   Only six of these, however, were microscopically diagnosed and one of these was a native from the Luangwa Valley, where sleeping sickness is known to occur.   The remaining five cases were natives travelling from the west towards the railway line.   It was in connection with these that the mine manager was anxious.   There are no tsetse fly on or near the mine and his only wish was to point .out that these travellers were repeatedly becoming infected by some focus on their route and to suggest that this route should be closed.

A close enquiry of the villagers and chiefs during the tour showed that these travellers invariably used one or other of four routes.   Two of these led through the game reserve and hence through or close to Areas I and II.   The other two routes led further south through the native reserve.

B.   Twenty-three Cases Collected by the Dispensary at Mumbwa, the Government Station.

This station had a small dispensary with four beds attached in ·medical charge of an African orderly.   The cases could be divided into four groups.

1. *Three Native Travellers from the West.*—They used the routes through the game reserve and Area I.   There are undoubtedly many other places where the Kafue River can be crossed, but the majority of natives used these two game reserve routes.   They could rely on employment on the way and they travelled along roads with villages at convenient stopping places.

2. *Eleven Cases from the Native Reserve.*—From the map it appears at first sight that these villages are a bad focus of infection, since the greatest number of the proved 1935 cases came from here.   There is no doubt that sleeping sickness is now endemic here but it will be noticed that the cases all occurred in villages bordering on the game reserve.   A close enquiry into the movements of these cases before they fell sick showed that they had all recently visited friends and relations in the game reserve villages.   The majority of them had been to Area I.   It was not, however, possible to question them all, as some had died.   The enquiry was complicated also by the fact that they were

reluctant to confess that they had been north for fear that trouble would arise over illicit shooting with their muzzle-loading guns in the game reserve.

The examination of recent movements of these cases therefore throws a definite pointer towards the game reserve, and particularly Area I, as an original source of infection.

Other suspects were reported but enquiry showed how unreliable native reports of this disease can be.

3. *Six Cases from Area I.*—This is a small group of ten villages in the eastern part of the game reserve. Six cases have been microscopically diagnosed during the year and the natives have reported twenty-five deaths. The tribe concerned here are the Bakaonde and are definitely more intelligent than some of their neighbours. They appeared to be more reliable in their knowledge of past cases, both as regards the symptoms and the manner of death. It was probable that a majority of the twenty-five suspected deaths had been actually due to sleeping sickness, though by no means all. The natives here were anxious about the disease, their villages were found to be badly cleared and the tsetse fly were numerous. This area was regarded as the chief focus of infection in the district.

4. *Three Cases from Area II.*—Area II is a scattered group of villages in the western part of the game reserve. Three cases were found here on the tour and there is no doubt that many others have occurred. The area is very remote. Part of it lies beyond the Kafue River and is outside the Hook. There is a great deal of game, including many large herds of buffalo; and the villages are already in the process of moving into the native reserve to the south. The villagers are waiting to reap their crops and are living in small collections of bad houses in uncleared parts of the bush. There is, moreover, frequent travelling between the old and new areas. Conditions are ideal for contracting sleeping sickness for the fly infestation is severe.

These conditions are temporary, and as soon as they are settled in their new site it is hoped that further infection will not occur. It seemed probable that this area was another focus of infection.

The result of these enquiries seemed to incriminate the villagers of the game reserve and the case was further strengthened by the fact that enquiry as to the movements of the cases of Areas I and II (*i.e.* game reserve villages) showed that they had done no travelling before they fell ill.

It is therefore certain that they, at any rate, contracted the disease at their own or neighbouring villages.

EVACUATION OF THE GAME RESERVE.

The remedy appeared to be the evacuation of the villages of Areas I and II combined with the usual grouping of the villages in contiguous areas, and clearing.

Both Areas I and II were under orders to move since it was already the intention of the administration to clear the whole game reserve of natives. Suitable new sites were, however, not easy to find. The whole area was infested with fly and the administrative difficulties were great since the offshoots of so many tribes were represented. Each small chief was reluctant to yield even a corner of his territory. From a medical point of view both areas should move to fly-free areas, but this was found to be impossible. The new areas (IA and IIA on the map) were finally decided upon after lengthy deliberation by the Chiefs, the Administration and the Medical Department.

Area IA is an empty, fertile tract of country only 25 miles from Mumbwa. There are tsetse fly here but in no great numbers and large areas are fly-free. There is a range of hills, the Kanchindu Hills, which lies between this new area and Mumbwa itself. These hills will act as a buffer between the two. They are rocky, barren, uninhabited and almost free of fly.

Area IIA is admittedly not an ideal site, but the administrative difficulties are great. The new villages have been sited along the banks of the Nangoma River in country which is fertile but fly-infested.

It was considered that both these new areas would be suitable and reasonably safe provided that the villages were well cleared and grouped together into small townships with adjoining cleared gardens so as to form continuous cleared areas. This has already been done successfully in sleeping sickness areas in this and other countries. Further measures advised have been the provision of two travelling orderlies to tour the newly sited villages for at least a year taking slides from the blood and glands of suspected cases ; and the enlargement of the Government dispensary at Mumbwa. By these means it was considered that the dangerous cases with trypanosomes in their blood could be removed at once to a fly-free area, and that the outbreak would be under control. The newly emptied game reserve could then be regarded as a buffer against any further spread of infection from the north, since hunting in it would be forbidden. The routes by which travellers from the west come to seek work would now be reduced to two. Those through the game reserve would be automatically closed and the natives diverted to the routes further south, since travellers will always choose a line of villages, which may employ them as they pass, rather than brave the desolation and increasing game of an evacuated area.

CONCLUSION.

We are now in a position to answer the questions raised by the District Administration.

1. *The endemicity or epidemicity of the disease* :

The disease is endemic. It is carried by *Glossina morsitans*. Nine cases existed in the area in December, 1935.

2. *The liability of travellers to infection and the possibility of control of the routes* :

This liability will always exist, both for Europeans and natives. The worst routes for natives pass through the game reserve and these will automatically be closed by the removal of all the villages. The Government stations further west should warn natives of the disease, and tell them that there are now no villages in the game reserve and that the area is closed. No cordon is necessary as the disease is not an epidemic.

3. *The desirability of opening the game reserve as a tourist attraction* :

This would be wrong until at least 10 years have elapsed.

4. *The question of evacuating the native reserve also* :

This is unnecessary. The cases found in the native reserve were probably, though not certainly, infected in the game reserve. Sleeping sickness is now endemic in northern villages of the native reserve, but the measures suggested should keep it in check and it is hoped eventually to reduce it to a very occasional case.

5. *Consideration of the best areas to which the natives now in the game reserve should be moved* :

After considerable discussion the areas marked Area IA and Area IIA have been chosen as most suitable under the circumstances.

6. *The best position for a native dispensary* :

This has been sited at the Chief's village in Area IIA. It could be reached by a branch road.

7. *The native cotton industry* :

This will be unaffected, provided that the west road is built.

8. *The advisability of building this contemplated west road through the sleeping sickness area* :

There is no reason why a road should not be built. Its course was sited during the tour of investigation so that it would pass through no villages and avoid infected areas. It should prove of immense value to the whole district. If the question is asked " Is there any danger to the Europeans and natives who build it ? " the answer is " Yes," but they could be protected to a certain extent by prophylactic doses of Bayer 205 and suitable clothing. The native labour could, moreover, be recruited locally and local natives would run no more danger than in their normal journeys from village to village. It is, of course, important to establish efficient fly-posts on the new road to prevent cars and lorries from carrying the tsetse fly into Mumbwa.

9. *The question of building an emergency landing ground and its best situation from the sleeping sickness point of view* :

This was sited near the pontoon which will carry the new west road over the Kafue River. A single village will be left there as caretakers of the river crossing. There have been no reports of sleeping sickness in this village and no cases were found there. There are no tsetse fly near and the surroundings of the village are clean and well cleared.

The above investigation is mainly of local importance, but it provides an illustration of one of the pressing health problems of Northern Rhodesia and records the finding of yet another area where sleeping sickness is endemic in Northern Rhodesia and not referred to in the standard works on tropical medicine.

A CASE OF BILHARZIAL MYELITIS.

BY

PROF. H. B. DAY

AND

DR. M. R. KENAWY.

From the Medical Unit, Cairo University.

Bilharziasis of the spinal cord is very rare. In a case of chronic myelitis under the care of one of us (H. B. D.) in 1911, bilharzia ova were found in the cord by the late Professor FERGUSON. In this case the bladder was the seat of chronic bilharzial disease. MÜLLER and STENDER (1930) recorded " A case of bilharziasis of the spinal cord simulating complete transverse thoracolumbar myelitis."

The present case was that of a boy aged 10 years who was brought to hospital on 16th November, 1935, with the complaint of sudden complete paralysis of the legs of 3 days' duration. The boy had been active the day before, but on awakening in the morning was unable to stand or to move his legs. No history of fever, sore throat or other antecedent disturbance was elicited.

On examination there was flaccid paralysis of both lower limbs with abolition of the knee and ankle jerks and no plantar reflex. There was retention of urine. The abdominal reflexes were active and no paralysis elsewhere was present. The affected muscles showed no wasting ; the thigh muscles gave a normal electrical response while those below the knee gave a weak normal response. Lumbar puncture showed no excess of pressure in the cerebrospinal fluid and the cell count was normal ; the albumin content was 30 mg. per cent. The fundi were healthy and the Wassermann reaction was negative both in the blood and spinal fluid. Nothing abnormal could be found in the chest, urine and stools. Blood count : hb. 80 per cent. ; reds 4,900,000 ; whites 8,000 ; polynuclear 70 ; lymphocytes 27 ; hyaline 2 ; eosinophile 1.

At first the case was looked on as a poliomyelitis, but two days later anaesthesia developed, first in the lateral aspects of the legs and then spreading up to the trunk. The retention was followed by incontinence of urine and faeces with the rapid development of bedsores. A septic parotitis with fever gave rise to pyaemia with fatal results.

Postmortem.[*]

Generalised septic infection. Liver and spleen were not enlarged and there was no evidence of bilharziasis in the urinary and alimentary tracts. No worms were found in the portal vein. Heart and lungs were normal.

[*]We wish to thank Prof. BERNARD SHAW of the Pathological Department for the pathological notes.

Spinal Cord.—" Over the lumbar enlargement the pia on the posterior surface is distinctly pink and the cord here feels softer than elsewhere. On serial sections of the cord in this region a group of four to five very minute necrotic areas appeared on the right side of the blind end of the anterior fissure. Further down in the lumbar enlargement similar areas were found on the left side. Nothing abnormal appeared in the brain."

Microscopic Examination.—" In the lumbar enlargement on either side in the depth of each of the anterior horns and in the angle between the anterior and posterior horns is a fair number of non-calcified *Bilharzia haematobium* ova, surrounded by bilharzial granulation tissue made up of histiocytes, lymphocytes and a few eosinophiles destroying and replacing the nervous tissue proper. Some of the ova are living, others are in process of absorption. The nerve cells show various degenerative changes and some of them show complete dissolution of the Nissl's granules and of the nuclei ; the dendrons also are disappearing. These affected nerve cells are quite close to the bilharzial ova, so that the nerve changes may be due to the diffusion of bilharzial toxins. In parts where there are no bilharzial ova the nerve cells look intact, contain the Nissl's granules and have their processes."

Section of cord showing bilharzia ovum surrounded by infiltration.

CONCLUSION.

(1) A case of bilharzial myelitis of acute onset is recorded.

(2) Attempts to find evidence of bilharzial infection of other tissues in this case have failed.

REFERENCE.

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THE INCIDENCE OF HYDATID DISEASE IN SYRIA.*

BY

EDWARD L. TURNER,
E. W. DENNIS
AND
I. KASSIS

From the Departments of Internal Medicine and Parasitology, American University of Beirut, Syria.

Hydatid disease is widespread throughout the world and is especially prevalent in those countries where sheep raising is a major industry. The disease is most common in Australia, New Zealand, South America, Iceland and the Balkan States. In all of these regions sheep raising is extensively engaged in and surveys have shown a high incidence of infection in man as well as in cattle and sheep. DEW (1928) has summarized the geographical distribution and incidence of hydatid disease in one of the chapters in his excellent monograph on the subject. The accompanying table, with modifications, outlines the information on incidence and distribution as given by him.

Although cases of hydatid disease had appeared frequently in hospital in-patients in Syria, no accurate data on the frequency of the infection appeared prior to the publications of GOODALE and KRISCHNER (1930). They examined 106 cattle in the abattoir of the city of Beirut and found that 44 (41·5 per cent.) of them had hydatid cysts in one or more sites. GOODALE (1931) reported finding ten positive Casoni reactions in a group of 112 consecutive tests on hospital patients. Half of these positives were confirmed by operation.

During the course of studies on the experimental immunization of the definitive and intermediate hosts against infection with *Echinococcus granulosus* a survey was undertaken of the incidence in Syria of hydatid disease in sheep, cattle and man. In a previous communication TURNER, BERBERIAN and DENNIS (1936) have reported the incidence of 20–25 per cent. intestinal infection with *E. granulosus* in dogs collected from the streets of Beirut. These figures were based on careful examination of the intestines of over 500 dogs.

The survey on the incidence of infection in sheep, goats, cattle and camels which is reported in this paper was undertaken during the summers of 1934 and 1935. The cities selected for the study were Beirut, Damascus, Homs and Aleppo. Beirut is located on the border of the Mediterranean Sea on the narrow plain west of the Lebanon Mountains. Damascus is situated on the western border of the great Syrian Desert about 110 km. from Beirut. Two mountain

ranges separate the two cities. Homs is also located on the western fringe of the Syrian Desert and is about 180 km. north of Damascus. Aleppo is located in the northern part of Syria only a short distance from the Turkish border and is about half-way between the Mediterranean Sea and the Euphrates River. All of the figures reported in this survey are the result of examinations made on animals slaughtered in the city abattoirs. The carcasses of all animals slaughtered in these cities are subjected to inspection before being stamped as suitable for meat. Each carcass with the viscera from the same animal was examined by us for the presence or absence of hydatid cysts at the time of the official inspection.

RESULTS.

(a) Incidence of hydatid disease in sheep, goats, cattle and camels.

At the city abattoir in Beirut, 1,156 sheep and goats were examined. Over one-fifth of the animals (22·1 per cent.) were infected with one or more readily detectable hydatid cysts. Two hundred and one cattle were examined and 45·1 per cent. found to have hydatid cysts.

In the Damascus abattoir 1,596 sheep and goats were examined and 28·5 per cent. found to be infected with hydatid disease. In this abattoir six camels were slaughtered for meat during the period when the survey was being conducted. All of the camels were found to have hydatid cysts in the viscera.

Outside of the littoral cities of the country very few cattle are used for meat. In the cities of the interior such as Damascus, Homs and Aleppo, etc., goats, sheep and occasionally camels, are the chief source of meat used by the population. In the city abattoir of Homs 551 sheep and goats were examined. Hydatid disease was present in 41·4 per cent. of the sheep and 13·8 per cent. of the goats. Five camels were slaughtered in Homs during the period of the survey. All of the camels were found to have hydatid cysts in the viscera.

In the abattoir of the city of Aleppo 1,126 sheep and goats were examined. Hydatid cysts were found in 27·8 per cent. of all the animals inspected. Four camels were examined at the same time and all of them were infected.

(b) Incidence of hydatid disease in hospital in-patients in Beirut.

During the 10-year period from April 1926 to April 1936, 14,057 cases were admitted to the medical, surgical and pediatric sections of the American University Hospital. Sixty-three, or one in every 223 of these patients was suffering from hydatid disease (see Table). The total number of patients admitted to all services including gynaecology and obstetrics, ophthalmology and otolaryngology was 24,500. This gives a ratio of one patient with hydatid disease to every 388 total hospital in-patients.

DISCUSSION.

The high incidence of infection of the definitive host with *Echinococcus granulosus* and of intermediate hosts with hydatid disease in Syria merits serious

TABLE.

GEOGRAPHICAL DISTRIBUTION AND INCIDENCE OF HYDATID DISEASE.

Figures given in the following table have been compiled from DEW (*Hydatid Disease*, 1928) ; BARNETT (1934) ; GOODALE and KRISCHNER (1931) ; WITENBERG (1934) ; and TURNER, DENNIS and KASSIS (1936).

Country.	Author and Date.	Summary of Observations.
ICELAND	GERAULT (1857) HJALTELIN (1869) KRABBE (1866) SAMBON (1925)	20 per cent. population infected. 16·6 per cent. autopsied bodies harboured cysts. 28·0 per cent. dogs harboured *E. granulosus*. With improvement of hygienic conditions incidence has fallen from 1 : 40 to 1 : 1,000 medical cases.
BULGARIA	DIKOFF (1925)	28·1 per cent. sheep 1 year old infected. 41·0 per cent. sheep over 1 year old infected. 11·0 per cent. goats infected. 16·0 per cent. oxen infected.
NORTH AFRICA	LAURIOL (1923) GALLI-VALERIO (1907) DEVE (1923) (and others)	State that 20 to 60 per cent. of sheep are infected and that there is a high incidence of infection in dogs and jackals.
EGYPT	MADDEN (1904) LOOS	1 : 1,000 surgical patients infected. There is a very low incidence of infection in dogs. No dogs infected in Cairo.
SOUTH AMERICA (Argentina)	VEGAS and CRANWELL (1902) CARBONELL and ZWANCK (1922) GREENWAY (1922)	4 per cent. sheep infected. 60 per cent. pigs infected. 50 per cent. sheep infected. 29·76 per cent. sheep infected. The number of hydatid cases in man quadrupled between 1901 and 1921.
SOUTH AFRICA	CRANKE	20 per cent. sheep infected.

NEW ZEALAND	THOMAS (1894)
	STIRLING (1897)
	HERCUS (1924)
	BARNETT (1934)
		Cysts in hospital in-patients 1 : 745 (1872-82).
		   ,, ,, ,, 1 : 529.
		   ,, ,, ,, 1 : 549 (1915-24).
		   ,, ,, ,, 1 : 362 (1915).
		   ,, ,, ,, 1 : 677 (1933).

AUSTRALIA	THOMAS (1894) STIRLING (1895) DEW (1928)	Incidence in hospital patients in Victoria 1 : 175. Incidence in hospital patients in South Australia 1 : 101. In Victoria hospital in-patients 1 : 159. Victoria hospital in-patients 1 : 294 (1915-24). South Australia hospital in-patients 1 : 388. New South Wales ,, ,, 1 : 345. Queensland ,, ,, 1 : 2,900. Tasmania ,, ,, 1 : 457. West Australia ,, ,, 1 : 1,000.
SYRIA	GOODALE and KRISCHNER (1930) TURNER, DENNIS and KASSIS (1936)	41·5 per cent. of cows examined in Beirut infected. 20 to 25 per cent. street dogs in Beirut infected with *E. granulosus*, 22·1 to 44·4 per cent. sheep infected, 13·8 to 27·8 per cent. goats infected, 45·7 per cent. cattle infected, 100 per cent. of camels examined were infected. 1 : 223 medical and surgical in-patients infected.
PALESTINE	WITENBERG (1934)	20 per cent. of stray dogs in Jerusalem harbour *E. granulosus*. 10 per cent. of jackals near Jerusalem infected.

consideration. Sheep, goat and camel raising are major industries in the interior of the country. Large camel herds roam the Syrian desert attended by Bedouin. Almost every camel herd is accompanied by flocks of sheep or goats and in every group there are always dogs. Dog droppings furnish a rich source of infection. Desert jackals, foxes and wolves probably also play a considerable role as definitive hosts. In the larger cities of the country great care is being taken to remove stray dogs from the streets.

It is of interest to note that of the fifteen camels examined all were found to be infected. This high incidence of infection is at least partially explained by the age factor. Camels are not brought to the city abattoirs for slaughter unless they have passed their period of usefulness. In the case of most of the camels in this group the owners had obviously brought their animals in for slaughter so that something might be realized on them before the animals died. Invariably these camels were many years older than the sheep, goats or cattle slaughtered in these abattoirs.

On one occasion during 1934 we were unable to obtain infected cattle viscera from the Beirut abattoir for several weeks. On investigation as to the probable cause of this sudden appearance of clean cattle it was learned that the animals had been shipped into the country from Europe.

The incidence of hydatid disease in medical and general surgical hospital in-patients in Syria is relatively quite as high as that reported by DEW and others (see table) in regions well known for the frequency of this disease.

CONCLUSIONS.

(1) A survey of the frequency of hydatid disease in sheep, goats, cattle and camels in Syria reveals a high incidence of infection in these intermediate hosts.

(2) The frequency of hydatid disease in man was 1 : 223 hospital admissions on the medical, surgical and pediatric services over the 10-year period from 1926-1936. For total hospital admissions during the same period the incidence of hydatid disease was 1 : 388.

(3) It seems to be indicated that careful control measures against the spread of hydatid disease in Syria are necessary.

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THE SIGNIFICANCE OF THE BUTTERFLY SIGN AND OF TONGUE PIGMENTATION.

BY

LEWIS J. McGREGOR*

*Medical Officer in charge, Hookworm and Malaria Branch,
Medical and Health Department, Mauritius.*

SINDERSON (1930) was the first to describe the " butterfly sign " as " an anomaly of pigmentation which constitutes a sign of definite diagnostic significance " and which he found present in about 20 per cent. of cases of schistosomiasis (*S. haematobium*) in Baghdad.

The body of the butterfly is represented by pigmentation of the bridge of the nose and the wings by patches extending on the cheeks from the sides of the nose. The pigmentation is painless, not raised above the skin surface, insidious in onset. The mark may be incomplete and may be reticulated but is usually evenly pigmented. SINDERSON attributes this pigmentation to the effect of intense solar rays on the most exposed part of the body, under the influence of a toxin generated by the parasite (*S. haematobium*).

In the course of anti-hookworm treatment in Mauritius I have noticed that this sign was common enough amongst the labouring classes. In negroes the sign is scarcely to be seen, but in persons of lighter complexion such as Indians and those of mixed race the sign is striking.

In Mauritius the distribution of schistosomiasis is strictly localized to certain regions and not very common, the total number of cases seen in the hospitals and dispensaries of the Colony being only 258 during the year 1932.

Hookworm disease, on the other hand, may be said to be evenly distributed and common for D'ARIFAT (1927-1931) found from 59·6 per cent. to 93·6 per cent. of the labouring classes infected in various localities.

I was consequently inclined to attribute the sign rather to hookworm disease than to schistosomiasis. In attempting to determine, in the course of anti-hookworm mass treatments in the field, the percentage of persons with the butterfly sign I noticed that a certain number with the butterfly sign had tongue pigmentation as well and that in rarer cases tongue pigmentation was present without the butterfly sign. The tongue pigmentation is seen as blue-black patches of varying size on the dorsum, tip or sides of the tongue.

Of 2,719 persons examined in the field, eighty-two or 3 per cent. had one of these signs. Of these sixty-eight or 2·5 per cent. had the butterfly sign alone

while fourteen or 0·5 per cent. had both the butterfly sign and tongue pigmentation and two or 0·07 per cent. had tongue pigmentation without the butterfly sign.

At the Hookworm Dispensary I instituted examination of the urine in addition to the routine examination of the stools in order to discover whether those with such abnormal pigmentations were infected with hookworms or with *Schistosoma haematobium*. The specimen of urine was taken immediately and examined microscopically after being centrifuged. The patients were relied upon to bring a specimen of stools each ; unfortunately many failed to do so. The stool specimens obtained were examined once only by Willis's method.

Of forty-two persons found with pigmentations, thirty-six had the butterfly sign and of these nineteen had tongue pigmentation in addition, while the other six had tongue pigmentation only. The examinations gave the following results :—

Number of stool specimens obtained	..	..	24
,, positive for hookworm	..	..	10 or 41·7 per cent.
,, ,, *Ascaris*	..	..	5 ,, 20·8 ,,
,, ,, *Trichocephalus*	..	..	14 ,, 58·3 ,,
,, ,, other worms	..	..	0
,, of urine specimens examined	..	..	42
,, with ova of *S. haematobium*	..	..	3 ,, 7·1 ,,
,, ,, evidence of cystitis only	..	..	27 ,, 64·1 ,,
,, ,, ,, renal inflammation only		1 ,, 2·4 ,,
,, ,, ,, cystitis and renal disease		11 ,, 26·2 ,,
,, ,, no evidence of urinary disease	..		0

Cystitis was diagnosed by the presence of numerous pus cells and transitional epithelial cells ; renal inflammation by the presence of casts, hyaline, granular or leucocytic.

I may add that these pigmentations were seen in adults only, thirty-five being women and seven men ; none of them showed acute symptoms.

The conclusion I draw from the above findings is that the butterfly sign and tongue pigmentation are not due to hookworm infestation as I thought, but may be caused by chronic infection of the urinary tract, not only due to *S. haematobium* but to bacteria as well, in dark skinned persons exposed to tropical sunlight.

SINDERSON's explanation seems, therefore, correct but incomplete.

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OBSERVATIONS ON LIVING *MICROFILARIA IMMITIS* IN THE CAPILLARY CIRCULATION OF BATS.

BY

DONALD L. AUGUSTINE, S.D.,

MADELEINE E. FIELD, PH.D.,

AND

CECIL K. DRINKER, M.D., S.D.

About one year ago the authors reported observations which showed that if blood heavily infected with *Microfilaria immitis* was given intravenously to an uninfected dog, microfilariae appeared in the lymph of the recipient in 10 minutes. Their appearance in lymph was not accompanied by haemorrhages and apparently the organisms made their way out of the blood capillary and into the lymph capillary without causing appreciable injury. We became interested in the manner in which these large organisms were able to leave blood capillaries without causing any evidence of damage, and at first attempted to observe this passage in the web of frogs injected with active *Microfilaria immitis*. The organisms, however, live but a short time in frog blood and these observations were completely unsatisfactory.

When the cold weather arrived it became possible to collect large numbers of the small brown bats which are plentiful in New England, and it was immediately found that the microfilariae lived in the bat's blood and remained entirely normal for many hours. Since it is possible to observe the blood capillaries in the wings of bats under very high powers of the microscope, material was then at hand in which it seemed that the microfilarial migration could be observed directly.

A typical experiment was as follows. A bat weighing 6 grammes was anaesthetized by intraperitoneal injection of nembutal. Shortly after, a concentrated suspension of *Microfilaria immitis* in 0·3 c.c. of a physiological saline

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solution was injected intravenously. The animal was then arranged for micro-scopical observation of the capillaries and lymphatics in the wing. Numerous microfilariae were at once seen in the capillaries and larger vessels. Frequently they occluded the capillaries and then worked their way through the stagnated column of blood cells and re-entered the active circulation. Active migration was more conspicuous than passive drifting along the vessels. Frequently organisms entered capillaries too small to pass through. They then backed out and made their way into larger blood vessels. Frequently even in the arterioles they moved against the blood stream, making slow progress by bracing them-selves through the crests of the alternate undulations of their bodies against the walls of the vessel. Pressure from the body undulations of the microfilariae does not alter the contours of the vessels—even the capillaries do not move when the organism passes along.

The anterior end of a microfilaria is constantly active in searching move-ments. As the blood vessels branch and become smaller the organisms may eventually enter capillaries which are obviously somewhat contracted and proceed into them until the lumen becomes too small, when they never were observed to escape or make permanent plugs. They simply backed out. Obser-vations were continued for as long as 4 hours, but microfilariae were never seen to leave the blood vessels nor were they found in the large lymphatics of the bat's wing.

These typical observations were a disappointment to the authors in that they failed to disclose the manner of migration of the microfilariae. They did, however, make clear one point which has often been puzzling, namely, the reason why microfilariae do not form emboli. Whenever these active organisms became stuck in a capillary they merely moved backwards against the current until they were safely on their way elsewhere.

LYNCH (1919)* made similar observations upon microfilaria immitis in an infected dog. He anaesthetized the animal, opened the abdomen, and observed the vessels in the omentum drawn over the stage of a microscope. He observed one organism which progressed against the stream in a small capillary. LYNCH's observations were confined to three organisms, and the circulation in the exposed omentum of the animal rapidly becomes highly abnormal. There is, however, no abnormality in the circulation of the bat's wing as observed by the authors, and there can be no question as to the validity of the observations recorded.

* LYNCH, K. M. (1919). Filarial periodicity. *J. Amer. med. Ass.* lxxiii, 760.

SOME OBSERVATIONS ON THE THERAPEUTICS OF MALARIA IN CEYLON.

BY

LIEUT.-COL. C. L. DUNN, C.I.E., D.P.H., I.M.S. (RETD.).

Late Special Malaria Officer and Director of Public Health, United Provinces, India.

FOREWORD.

These observations are based on an enquiry into the malaria epidemic in Ceylon of 1934-35, carried out by me over a period of 9 weeks from 27th October, 1935, with the support of the Colonial Office and the London School of Hygiene and Tropical Medicine. I received every possible assistance from the Government of Ceylon in the Ministry of Health, and of the officers of the Medical and Sanitary Departments, and had complete access to all Government files and publications connected with the subject.

I received great personal help and consideration from Dr. R. BRIERCLIFFE, C.M.G., O.B.E., Director, Medical and Sanitary Services, from Mr. CARTER, Medical Entomologist and from Lt.-Col. W. W. CLEMESHA, C.I.E., I.M.S. (retd.), Malariologist to the Ceylon Estates Proprietary Association, and made extensive tours in the island in the company of these and other officers. Without their help and co-operation it would not have been possible for me to gain the extensive information I did in this short period.

1.—Therapeutic Measures used by Government Agency.

The routine treatment for malaria in the Government Hospitals in Ceylon is the administration of a stock mixture containing $7\frac{1}{2}$ grains of quinine bisulphate per dose. This mixture is given three times daily until the febrile symptoms have disappeared and for as many more days as the patient can be persuaded to remain in hospital; and 2 days' further supply is handed to each patient on leaving hospital. The great majority of patients, however, left hospital as soon as they felt well, and did not continue the treatment. The average stay in hospital under this treatment was between 6 and 7 days.

Serious cases were given intravenous and intramuscular injections when considered necessary by the physicians.

In addition to quinine, large quantities of plasmoquine were issued and used, generally in conjunction with varying amounts of quinine.

Atebrin was also issued and used, chiefly in hospitals, but some attempts at mass treatment with this drug were tried.

Atebrin musonate supplied by the makers, on payment, was also tried in hospitals under test conditions as far as possible ; and was also tried in the field.

I also came across a few instances of other therapeutic agents being used, such as esanofele, tebetren, etc., but these instances were so few as to be negligible.

Combinations of plasmoquine and quinine, atebrin and quinine and plasmoquine and atebrin were also tried in some hospitals and their effects observed.

2.—Therapeutic Measures used by the Ceylon Estates Proprietary Association.

Out of about 1,300 tea, rubber and cocoanut estates which subscribe to the above Association, about 560 subscribe to the Malaria Control Scheme of the Association, a very high percentage of the estates situated in the malarial zone., These estates cover an area of 420,000 acres, and are populated by about 300,000 Tamil coolies and their dependants. The expert adviser of the Malaria Control Committee is Lt.-Col. W. W. Clemesha, who has been 6 years in the employ of the Association.

Clemesha, as the result of many years' experience in India and Ceylon, applies a standard treatment to all the adult population of these estates, which consists of quinine bisulphate or quinine bihydrochloride 30 grains until the febrile symptoms subside ; or, in some estates, the same quantity of cinchona febrifuge. Children under 10 years are getting a standard treatment of euquinine and milk, with a dosage according to age. The quinine treatment is followed up among adults and children over 10, by $9\frac{1}{2}$ grains of quinine and $\frac{1}{6}$ grain of

plasmoquine daily for 6 days. For 14 days tonic iron pills are given, and the quinine-plasmoquine treatment again given for a further 6 days.

CLEMESHA's treatment is an attempt to cure each clinical attack of malaria as it arises, and to eliminate human carriers of the gametocytes of *Plasmodium falciparum.*

CLEMESHA also carried out one interesting experiment with atebrin on the Halvatura Estate. From 1st December, 1934, until 11th June, 1935, all cases of malaria were treated with 3 grains of atebrin daily. During this period, 328 cases occurred, but a large number only had partial treatment. 106 cases, however, received 5 consecutive days' treatment (15 grains of atebrin) between 1st December, 1934, and 1st March, 1935 ; and were still employed by the Estate on 1st June, 1935. Of these, fifty-six relapsed. The relapse rate was thus 52·8 per cent. The dosage however, was two-thirds the amount recommended by the makers and this large relapse rate would appear to be due to insufficient dosage.

3.—RESULTS OBTAINED WITH THE VARIOUS TREATMENTS USED.

It is obvious that during the course of an epidemic of such devastating proportions as that which occurred in Ceylon, there were almost insuperable difficulties in any attempts to carry out controlled comparative experiments with the therapeutic agents employed. CLEMESHA only made the one referred to in the previous paragraph, but BRIERCLIFFE (1933) and his staff carried out several experiments in order to ascertain whether the wholesale use of the synthetic remedies would not be more efficacious than quinine in cutting short the epidemic, especially in view of the success reported with the extensive use of atebrin in Malaya by HOOPS (1933), BARROWMAN (1933) and DUNCAN (1933). BRIERCLIFFE tried no experiments with combinations of quinine and plasmoquine, the success of which has been reported by several observers.

Atebrin Tablets per os.

179,000 tablets of atebrin were issued to the hospitals and dispensaries, and medical officers were asked to use this drug and observe its effects. The dosage recommended by the makers, 1½ grains three times a day after food for 5 days, was employed. After extensive enquiries in the hospitals and dispensaries and an examination of the hospital returns, I found that it was only possible to arrive at general impressions as to the value of this treatment. It was impossible to differentiate between relapses and reinfections when the hospital books showed that patients had returned for second and third courses of treatment, and in many cases, the courses of treatment were incomplete owing to patients leaving hospital and ceasing to attend dispensaries as out-patients when the febrile symptoms subsided. From a study of the

hospital registers, however, the following conclusions may be regarded as fairly accurate.

Control of Febrile Attacks.

In the epidemic area in Ceylon, atebrin took longer to control the febrile attack than quinine, the average time being between 2 and 3 days as against 1½ days.

Relapses.

Patients treated with atebrin appeared to have fewer relapses or re-infections than with quinine.

Toxicity.

Medical officers recorded their opinions, that there was a frequency of abdominal discomfort and even great pain. Atebrin was considered unsuitable for children owing to the high percentage of cases in which vomiting and convulsions were induced. Discoloration of the skin was rare and has not raised any barrier to the use of this drug in Ceylon. Symptoms of mental derangement were fairly frequent. These symptoms usually lasted for 2 or 3 days. The number of such cases observed among 792 patients at three dispensaries, was eight, or 1 per cent.

Atebrin and Quinine.

If these two drugs were given together, there appeared to be a greater incidence of toxic symptoms, but if given consecutively, these symptoms were less in evidence. No clinical advantage in this combination was observed.

Atebrin and Plasmoquine.

A combination of these drugs seemed to produce a high degree of toxicity even when the plasmoquine component was as little as ½-grain per diem for 5 days. The toxicity of the combination was higher than that of either drug used alone.

Quinine and Plasmoquine.

As given consecutively by CLEMESHA (page 234) produced no toxic symptoms worth recording. CLEMESHA (1935) gives many results of his standard treatment, and certainly these results afford a strong testimony of the efficacy of his treatment as compared with the results obtained in the same areas with the routine quinine treatment.

Plasmoquine Simplex.

I found no record of this treatment being used at any of the hospitals or dispensaries in Ceylon.

Atebrin Musonate.

BRIERCLIFFE (1935) supervised some tests of the efficacy of injections of this drug, in April and May, 1935, as compared with two injections of quinine bihydrochloride followed by quinine by the mouth. The dosage of atebrin musonate given was that recommended by the makers, *i.e.* 0·375 grammes on 2 consecutive days. Quinine bihydrochloride 15 grains, was given to the control cases on 2 consecutive days, followed by 22½ grains of quinine bisulphate mixture for 6 days. The tests were carried out on 1,105 hospital patients in twelve hospitals : 681 patients were treated with atebrin musonate, and 424 with quinine. The two series of cases were strictly comparable as to type of case.

The result observed was the *length of time taken to stabilize the temperature*, among males and females, in cases of benign tertian and malignant tertian fever, and BRIERCLIFFE gives complete tables of the results recorded. These briefly were that both drugs stablized the temperature in about 81 per cent. of the cases within 6 days, and that the atebrin musonate stabilized the temperature on the average about 9 hours sooner than the quinine treatment. The average for atebrin was 61½ hours, and for quinine, 70½ hours. Cases of benign tertian fever were stablized on the average about 16 hours sooner than malignant tertian cases.

The *toxicity* of both treatments was also observed.

Among the 623 atebrin musonate cases, there were twenty-three cases or 3·4 per cent. of cases of vomiting or abdominal pain, as compared with one case, 0·2 per cent. with quinine ; thirteen cases or 1·9 per cent. of mental derangement as compared with one or 0·2 per cent. ; two cases or 0·3 per cent. of collapse as compared with nil ; and four deaths or 0·6 per cent. as compared with nil.

BRIERCLIFFE gives full details of the postmortem examination of the deaths, which establish beyond doubt that the deaths were due to atebrin poisoning. Atebrin could not be recovered from the kidneys or urine in these cases. It is therefore concluded that atebrin poisoning supervenes if the kidneys fail to eliminate the drug from the blood.

The *relapse* rate could not be observed as the stay in hospital was so short, but Dr. P. B. FERNANDO of the Colombo General Hospital observed seventy patients who, for a minimum of 3 weeks, were kept in hospital where the risk of re-infection was negligible. Of these, twenty cases relapsed. Dr. FERNANDO only included among his cases of relapse patients who had both a return of fever and of parasites in the blood. Risk of infection was negligible.

Dr. C. G. HOOLE treated twenty policemen with atebrin musonate ; two had severe toxic symptoms, and ten relapsed within a month. Risk of infection negligible.

Dr. S. DE SILVA treated eighteen policemen in the Mahara jail, and eight relapsed in a month. Risk of infection slight.

In Kurunegala, sixty-five of the hospital staff were treated by Dr. SIMEONS with atebrin musonate and only seven relapsed in 6 weeks, but none of these were suffering from fever when injected.

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Field Treatment with Atebrin Musonate.

In May, 1935, 1,387 persons in three groups of malarious villages received atebrin musonate treatment as an experiment. The cases were carefully selected, all suffering from nephritis, severe hookworm infection, oedema, advanced debility and anaemia being excluded. Of the cases followed up, 7 per cent. had febrile relapses in 14 days, but in 117 of these cases, where blood films were taken, forty-nine had parasitic relapses or 41·8 per cent.

Among these there were few toxic cases, but fifty-three cases had nausea, vomiting, collapse, etc., and there were eight cases of mental derangement ; one had to be kept in a strait jacket, five were detained for more than a· week, and one died of exhaustion from mania. Many had painful swellings and seven developed abscesses.

The expense of this experiment worked out at Rs.4·24 per head treated. The treatment was most unpopular with the villagers owing chiefly to the mental symptoms caused in a small number of cases.

Other Experiments with Atebrin Musonate.

Prior to BRIERCLIFFE's tests, great success in effecting a rapid clinical cure with atebrin musonate had been recorded in a series of twenty-one cases by BLAZE and SIMEONS (1935) and about 300 people were treated by medical officers in collaboration with Dr. SIMEONS with success in controlling the symptoms, but no record was kept in any of these cases of any untoward happenings, and the relapse rate also could not be observed.

In addition, several other medical officers tried the treatment in their hospitals, and although the therapeutic effects were usually good, untoward effects of many kinds are referred to by BRIERCLIFFE as having occurred, such as sudden deaths among children, a few minutes to a few hours after injections, collapse, convulsions, etc. Epileptiform convulsions are stated to have been extremely rare, only occurring about " once in every 900 to 1,000 treatments " (BRIERCLIFFE, 1935) ; but deaths preceded by collapse probably occurred more than once in every 200 cases. BRIERCLIFFE's conclusions on the merits and demerits of the drugs used in the circumstances and under the conditions, *i.e.* during the progress of a severe epidemic, are briefly as follows :—

(1) Atebrin musonate is as effective as quinine in controlling symptoms. Atebrin by the mouth is almost equally effective.

(2) It was not possible to arrive at definite conclusions as to the relapse rate, but the impression is gained that 5 days' treatment with atebrin by the mouth is more effective in preventing relapses than a week's treatment with quinine in the Ceylon standard dosage.

(3) Pain and swelling at the site of injection occurs after atebrin, but not so frequently as after quinine.

(4) Minor toxic manifestations occurred in a small percentage of patients after atebrin injections, but did not occur so frequently as after atebrin by the mouth.

(5) The temporary mental derangement which may occur after atebrin, is a very serious objection to the use of this drug.

(6) The greatest care is necessary for the selection of patients for treatment with atebrin musonate. Probably rather more than $\frac{1}{2}$ per cent. of hospital patients treated with this drug, have died from its effects.

(7) The manufacturers state that there are no contra-indications, but experience in Ceylon indicates that atebrin treatment *per os* or by injection is definitely contra-indicated where there is any disturbance of the kidneys. Chronic poisoning appears to be associated with imperfect excretion of the drug. Small children appear to be specially liable to collapse and convulsions.

(8) Medical officers may ask " when should atebrin injections be used ? " The majority of patients do not require injections of any drug, but if injections are indicated, quinine is to be preferred because of its greater safety.

4.—Discussion of the Results of Atebrin Treatments.

As the result of my own investigations and the perusal of the literature on the subject, I am in full agreement with Briercliffe's general conclusions. Atebrin appears to be unsuitable for mass treatment, but is certainly a useful addition to our list of therapeutic agents for use in hospital practice when quinine is found less suitable, or ineffective. As Green (1934) says, " Is it justifiable in dealing with a condition, which almost always responds readily to quinine, to employ instead, in the expectation of lessening the tendency to relapse, a drug of similar potency but somewhat uncertain toxicity ? "

This remark of Green's is in my opinion the most logical conclusion that can be arrived at by a study of the results obtained with atebrin treatment. Green arrived at this conclusion in spite of the highly encouraging results reported by the Malayan workers, results which, as regards toxicity and relapse rates when compared with those obtained in Ceylon, are much more favourable to atebrin therapy. Green's opinion is supported by Kingsbury (1934) who describes cases of mental disturbance and minor untoward effects in Malaya. The low relapse rate obtained in Malaya has not been approached in any other country with atebrin alone, but lower rates have been obtained there by combinations of atebrin and plasmoquine, and in the tests carried out by the Army in India which will be published shortly. Morishita, Miyahara and Isioka (1934) on the contrary, report a relapse rate of 50 per cent. using the standard atebrin *per os* treatment and Komp and Clark (1934) working in Panama in controlled villages for a period of 8 months, found the parasite rate only reduced

from 21·6 to 18·6 per cent. The results obtained with atebrin musonate in controlling the febrile attack slightly more quickly than with quinine bihydro-chloride injections have recently been more or less confirmed by FIELD and NIVEN (1936) working in Malaya. They treated 286 cases of acute malaria due to *P. falciparum*, *P. vivax* and *P. malariae* alternately with two atebrin musonate injections and quinine bihydrochloride administered orally for 7 days, the dosage being in accordance with the body weight. The fever in malignant tertian cases was stabilised in about 5 days with both treatments, but in benign tertian cases, the atebrin stabilised the temperature in an appreciably shorter period. Two toxic cases (epileptic fits), one of which was severe, occurred among the atebrin cases. The relapse rate could not be observed but an analysis of the cases returning to hospital within 10 weeks, suggested that relapses after atebrin musonate are fairly common. CARMAN and CORMACK (1936) working in Kenya, treated sixty-eight cases of which sixty-six were malignant tertian fever in a controlled experiment in which every alternate case received one to three intravenous or intramuscular injections of atebrin musonate plus 0·01 gramme of plasmoquine three times daily for 5 days ; and the others with 30 grains of quinine bihydrochloride daily plus 0·01 gramme plasmoquine for 5 days ; but no hard and fast rule was observed, and injections were also given as the severity of the case indicated. In all the cases the temperature returned to normal within 5 days, but the atebrin cases were controlled slightly more quickly, the percentage of cases showing asexual parasites was much less with the quinine-treated cases, and very much less in the case of sexual parasites.

It is surprising in view of the results obtained by other workers, that no toxicity was observed in either series with these comparatively large doses of plasmoquine.

The toxicity of combinations of atebrin and plasmoquine has been reported by many workers in India. These workers, as well as FIELD and NIVEN, emphasize the high cost of atebrin musonate and remark on its consequent unsuitability for routine use among natives.

5.—DISCUSSION OF THE RESULTS OF QUININE AND PLASMOQUINE TREATMENT.

As noted above, this treatment was used extensively by CLEMESHA among the populations of the tea, rubber and cocoanut estates under the Malaria Control Scheme, and in his report he records the success he has obtained over a series of years, in reducing the incidence of malaria by very large percentages among the people treated, and has compared this reduction with the high incidence of malaria in adjacent comparable populations. The success of this combination has been reported at various times, by SINTON and a large number of other workers in the Army in India, and in Bengal, and the United Provinces, in the Annual Report of the Directors of Public Health. A combination of

these drugs has also been adopted by the United Fruit Company of America. CLEMESHA's treatment is directed at curing the primary febrile attack clinically with quinine, reducing the relapse rate by a follow-up treatment of quinine and plasmoquine, and eliminating the sexual forms, which—in his experience and that of others—are prone to appear in the peripheral blood after the 7th day, and a further course of quinine and plasmoquine at a time when he has been led to expect a later appearance of parasites, sexual and asexual. Although CLEMESHA was unable to show me direct proof of his theories in this respect, his opinions must be treated with respect in view of his wide experience in treating thousands of tea garden coolies in India and Ceylon for many years. At my request he is this year going to carry out some controlled comparative experiments with quinine, and with quinine and plasmoquine, in comparable estates ; while BRIERCLIFFE is carrying out similar experiments in all parts of Ceylon, alternate admissions to the jail hospitals being used for the experiment. The treatment will be 3 days quinine 30 grains and plasmoquine 0·02 gramme daily, as compared with quinine bisulphate 22½ grains daily for 7 days. This very short course of quinine and plasmoquine is being tried in order to ascertain whether such a short course will be reasonably effective, as it is necessary to endeavour to apply the shortest effective course to mass therapy in the tropics, owing to the difficulties met with in endeavouring to persuade patients to continue treatment after the febrile symptoms have subsided. The treatment was that used extensively under my direction in the United Provinces in 1931 and 1932 as the result of controlled experiments. In one experiment carried out in twenty villages, 856 cases were treated in 4 months with a febrile relapse rate of 8 per cent. In another, carried out in the Central and District Jails, Lucknow, at the same time, 734 cases were treated with a febrile relapse rate of 15·2 per cent. The control in reporting relapses in the latter experiment, was unimpeachable, which it naturally could not be in the villages. This treatment is, as far as I am aware, the shortest treatment yet recommended and extensively used for malarial therapy. The United Fruit Company of America use a standard treatment of 24 grains of quinine and 0·02 gramme of plasmoquine daily for 6 days as a routine treatment. SINTON and his colleagues (1930) used a dosage of 20 grains of quinine and 0·06 and 0·04 grammes of plasmoquine for 21 days, but this course was specially directed against chronic relapsing cases of benign tertian fever, when other treatments had failed. With the latter treatment the relapse rate was 8·4 per cent. Other Army workers with similar dosage for a similar period among patients admitted to the military hospitals, have recorded relapse rates between 4·1 and 8 per cent. MORISHITA, MIYAHARA and ISIOKA (1934) record a relapse rate of 12·9 per cent. with this combination, but these were parasitic relapses observed over 8 weeks, while the Army in India results are expressed as febrile relapses only.

PETER (1935) states that this combination gives a lower relapse rate than any other therapeutic measure. I do not think that this short 3-day course will

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5.—DISCUSSION OF THE RESULTS OF QUININE AND PLASMOQUINE TREATMENT.

As noted above, this treatment was used extensively by CLEMESHA among the populations of the tea, rubber and cocoanut estates under the Malaria Control Scheme, and in his report he records the success he has obtained over a series of years, in reducing the incidence of malaria by very large percentages among the people treated, and has compared this reduction with the high incidence of malaria in adjacent comparable populations. The success of this combination has been reported at various times, by SINTON and a large number of other workers in the Army in India, and in Bengal, and the United Provinces, in the Annual Report of the Directors of Public Health. A combination of

these drugs has also been adopted by the United Fruit Company of America. CLEMESHA's treatment is directed at curing the primary febrile attack clinically with quinine, reducing the relapse rate by a follow-up treatment of quinine and plasmoquine, and eliminating the sexual forms, which—in his experience and that of others—are prone to appear in the peripheral blood after the 7th day, and a further course of quinine and plasmoquine at a time when he has been led to expect a later appearance of parasites, sexual and asexual. Although CLEMESHA was unable to show me direct proof of his theories in this respect, his opinions must be treated with respect in view of his wide experience in treating thousands of tea garden coolies in India and Ceylon for many years. At my request he is this year going to carry out some controlled comparative experiments with quinine, and with quinine and plasmoquine, in comparable estates ; while BRIERCLIFFE is carrying out similar experiments in all parts of Ceylon, alternate admissions to the jail hospitals being used for the experiment. The treatment will be 3 days quinine 30 grains and plasmoquine 0·02 gramme daily, as compared with quinine bisulphate 22½ grains daily for 7 days. This very short course of quinine and plasmoquine is being tried in order to ascertain whether such a short course will be reasonably effective, as it is necessary to endeavour to apply the shortest effective course to mass therapy in the tropics, owing to the difficulties met with in endeavouring to persuade patients to continue treatment after the febrile symptoms have subsided. The treatment was that used extensively under my direction in the United Provinces in 1931 and 1932 as the result of controlled experiments. In one experiment carried out in twenty villages, 856 cases were treated in 4 months with a febrile relapse rate of 8 per cent. In another, carried out in the Central and District Jails, Lucknow, at the same time, 734 cases were treated with a febrile relapse rate of 15·2 per cent. The control in reporting relapses in the latter experiment, was unimpeachable, which it naturally could not be in the villages. This treatment is, as far as I am aware, the shortest treatment yet recommended and extensively used for malarial therapy. The United Fruit Company of America use a standard treatment of 24 grains of quinine and 0·02 gramme of plasmoquine daily for 6 days as a routine treatment. SINTON and his colleagues (1930) used a dosage of 20 grains of quinine and 0·06 and 0·04 grammes of plasmoquine for 21 days, but this course was specially directed against chronic relapsing cases of benign tertian fever, when other treatments had failed. With the latter treatment the relapse rate was 8·4 per cent. Other Army workers with similar dosage for a similar period among patients admitted to the military hospitals, have recorded relapse rates between 4·1 and 8 per cent. MORISHITA, MIYAHARA and ISIOKA (1934) record a relapse rate of 12·9 per cent. with this combination, but these were parasitic relapses observed over 8 weeks, while the Army in India results are expressed as febrile relapses only.

PETER (1935) states that this combination gives a lower relapse rate than any other therapeutic measure. I do not think that this short 3-day course will

be found highly effective in " preventing relapses," which is, after all, another way of expressing " effecting a radical cure," nor in preventing the spread of the disease by destroying the viability of the gametocytes as they are not necessarily to be found in the peripheral blood during the febrile attack. It will, however, in nearly all cases effect a clinical cure and allow the patient to return to work. It will also conform to the policy advocated by the Malaria Commission of the League of Nations (1933) in dealing with mass therapy, and assist the patient to acquire " tolerance " and " immunity." I do not think that this treatment will be sufficient to employ in a " highly susceptible " population with no acquired " tolerance " ; but for mass treatment among the " tolerant " populations of the malarious tracts of India and Ceylon, it will be adequate.

Some doubts have been expressed with regard to the danger of using plasmoquine in mass treatment. In my experience, in the dosage recommended, it is perfectly safe. CLEMESHA has had the same experience, and so has SUR (1932) in Bengal, and the workers under the United Fruit Company of America. It is only when doses of over 0·02 gramme per day are used, that serious toxic symptoms are recorded.

I am aware that there is a great diversity of opinion on the efficacy of combinations of quinine and plasmoquine. The Malaria Commission pronounced definitely against this combination, but expressed themselves prepared to review their attitude if the efficacy of this combination in non-toxic doses could be proved by clinical trials on induced malaria in man. I think the recorded evidence of workers from most countries is favourable to its use, especially in malarious countries for mass treatment. In primary outbreaks in a " susceptible " population, a more prolonged treatment will be required to effect a clinical cure, but even in such cases a 6-day treatment should be sufficient.

It is not contended that this treatment will suffice in severe cases with a heavy infestation of parasites, or in algid and cerebral cases. Such cases, which are usually the result of incorrect treatment or no treatment at all, must, of course, be dealt with on their merits with more drastic remedies.

It is also not contended that this treatment will be equally effective in all malarious countries. It is well known that the virulence of the strains of parasites varies greatly in different countries, which accounts for the failure of this treatment in the hands of SWELLENGREBEL and DE BUCK (1932) on patients infested with multiple bites, and the Madagascar strain of parasite. But in India and in Ceylon, this treatment has been found effective as well as in America, and it is thus on account of the ease with which it can be distributed, its safety and cheapness, to be preferred to quinine alone or to atebrin alone, or in combination with other therapeutic agents.

A combination of quinine and plasmoquine is more effective than quinine alone, and adds little to the expense of a treatment. It is safer than atebrin *per os*, half the cost and more effective.

6.—SUMMARY OF CONCLUSIONS.

1. Quinine in solution was found to be the safest and most effective remedy for the mass therapeutics of malaria in Ceylon by the Medical Department, the course of treatment aimed at being $22\frac{1}{2}$ grains daily for 7 days.

2. In the Ceylon Estates Proprietary Association's Malaria Control Scheme, a follow-up course of quinine and plasmoquine for two periods of 6 days each, after the febrile symptoms had been controlled by quinine, was found an effective standard treatment.

3. Atebrin *per os* for 5 days in $1\frac{1}{2}$-grain tablets given three times a day, was found effective in controlling the febrile symptoms, and in reducing the relapse rate, but a small percentage of untoward symptoms such as abnormal disturbances and varying degrees of mental derangement among adults, and collapse and convulsions among children, led the officers of the Medical Department to form the opinion that it was unsuitable for mass distribution, but was useful in selected cases for hospital treatment under medical supervision.

It was also found that a treatment with atebrin is $2\frac{1}{2}$ times more expensive than a routine treatment with quinine.

4. Atebrin musonate was found to be most effective in controlling the febrile symptoms, but was found unsuitable for administration except to carefully selected cases, owing to its toxicity in a small percentage of cases. It is quite unsuitable for mass therapy owing to the expense of its administration and cost, and its comparatively high accident rate.

5. Combinations of atebrin and quinine, and atebrin and plasmoquine, were found to be more toxic in their effects than when given alone. If given consecutively, the toxicity was reduced.

6. Atebrin in any form cannot, therefore, take the place of quinine, which in the vast majority of cases, is effective in producing a clinical cure, and often a radical cure, especially in mass therapy.

7. From the results obtained by the use of a combination of quinine and plasmoquine in short courses, and in non-toxic dosage, this would appear to be the best agent to use in mass therapy for all types of parasites, and in all their stages. Its usefulness has been proved in India and America, and it has given good results except when used on patients infested with virulent strains of parasites. In Ceylon, where none of the parasites found have shown any abnormal virulence, it should give good results in short courses among " tolerant " populations, and in longer courses among " susceptible " populations.

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THE SEASONAL INFECTIVITY OF MOSQUITOES AS DETERMINED BY A STUDY OF THE INCIDENCE OF INFANTILE MALARIA.

BY

C. STRICKLAND, M.A., M.D.,

Professor of Medical Entomology, School of Tropical Medicine, Calcutta,

AND

S. C. SEN GUPTA, D.T.M.,

Resident Medical Officer, Hope Tea Estate, North Bengal.

The period during which malarial infection is being conveyed by the mosquito to man is, of course, that during which certain types of prophylactic efforts should be most concentrated. Any evidence indicating the season of inoculation is therefore important.

One must, however, issue a *caveat* against the view that the rate of inoculation by the mosquito is necessarily at its highest when the incidence of the disease in man is greatest ; there are for instance the reports from Holland of autumnal inoculation and of vernal epidemic.

It must also be noted that the infection rate in the mosquito, as determined either in the laboratory or in nature, may be a fallacious guide, since it does not follow that the inoculation of man is in proportion to the rate of infection in the mosquito; mosquitoes may indeed become heavily infected at a season when other factors apparently tend to nullify their potential danger to man. STRICKLAND and ROY (1933) found that *Anopheles stephensi* in Calcutta was infectible to the extent of about 100 per cent. in the laboratory during the cold weather months when the epidemic of malaria was rapidly abating.

But apart from such considerations the acquisition of the data concerning infection in the mosquito is a troublesome and even expensive business, especially as the results cannot with certainty be regarded as having much more than a local applicability. To determine the infection rates obtainable in the laboratory a well-equipped institute is almost essential, while to ascertain the rates in wild mosquitoes is a very costly undertaking. Thus STRICKLAND and CHOWDHURY (1933) dealt with about 26,000 anophelines in about 5 months, the enquiry costing about Rs. 8150/- (or about £600, English currency), and TIMBRES (1935) dissected 73,873 mosquitoes over about 18 months in order to discover 148 infections.

This note, therefore, on a practical and cheaper method of determining the seasonal liability to infection may be welcomed by many of those engaged in malaria work.

The material required for a given locality is a record of all infants· born during the course of at least 1 year, with the date of birth of each child and date of its first attack of malaria.

Assuming that the incubation period of malaria in an infant is at least 10 days* infection must have taken place at some time between the date of its birth and a date 10 days before the first onset of fever.

An infant developing fever a year after birth may, however, have had either a short or a long incubation period ; the infection may indeed even have been inoculated on the very day of its birth. As a result, however, of a scrutiny of the records one may prove that some cases of infection have taken place in the same month as birth ; and, while in other cases perhaps the interpretation may be equivocal, in general it may be stated that valuable information will be obtained as to the period within which infections in the infant population must have occurred. It must not be forgotten, however, that the greater the number of records available the more complete and precise will be the information obtained.

As an example of the method we may consider the data obtained from an enquiry in the Duars of North Bengal. This enquiry was ancillary to a malaria survey by the senior writer made some time ago and was undertaken at the request of the Indian Tea Association. It had the advantage of the collaboration of the tea garden medical officers (STRICKLAND et al, 1927).

These data relate to births during 1934 and 1935 on Hope Tea Estate, of which the junior author is the resident doctor. On this estate certain maternity benefits, as well as the general administration, ensure the continued observation of mother and infant, so that the records shown below of the date of birth of the infants and of any illness, may be regarded with every confidence. The diagnosis of malaria was in all cases confirmed by the microscope.

*After the completion of the analysis, reported in Table I, the following record has become available : 1936, No. 12, born 7th April, date of first fever 14th April (diagnosed as malaria under the microscope).

A preliminary analysis of the data is given in Table I. It shows that all the children, about whom data are available, developed malaria during the first year of life.

TABLE I.

	1934.	1935.	Total.
Born on the estate	56	66	122
Omitted from the analysis because of death, etc., within a year	11	11	22
Remaining	45	55	100
Number developing malaria within a year of birth	45	55	100
Percentage ditto.	100	100	100

From an examination of the data given in Table II it is clear that, in the Bengal Duars, anopheline infectivity and infant inoculation are possible in the months April, September, October and November, and in one or other of the

TABLE II.

Ref. No.	Month of Birth.	Date of Birth.	Number of Days before Onset of Fever.	Month in which Infection Occurred if Incubation Period was			Months of possible Infection.
				20 Days.	15 Days.	10 Days.	
4	January	11. 1.36	40	February	February	February	January & February
10	February	11. 2.35	23	February	March	March	February & March
7	March	11. 3.36	31	March	March	April	March & April
12	April	7. 4.36	7	April	April	April	April only
3	May	12. 5.35	49	June	June	June	May & June
17	June	10. 6.35	42	July	July	July	June & July
31	July	21. 7.34	39	August	August	August	July & August
20		3. 7.35	46	July	August	August	July & August
28	August	19. 8.35	30	August	September	September	August & September
36		7. 8.34	36	August	August	September	August & September
34	September	4. 9.35	16	September	September	September	September only
43	October	8.10.35	28	October	October	October	October only
51	November	4.11.35	29	November	November	November	November only
Jiti*	December	5.12.35	63	January	January	January	December & January

*Child from an adjoining estate.

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months January or February, February or March, May or June, June or July, July or August, or indeed in all of these months.

If this analysis had not thus unequivocally indicated that inoculation may take place at least in alternate months throughout the year, one might have concluded that the first attack of malaria in an older infant was an instance of

TABLE III.

INOCULATIONS NOT MORE THAN 60 DAYS AFTER BIRTH.

Month of Births.	Year of Births.	Number of Births.	Number of Inocula-tions.	Total for 2 Years in months.		Seasonal Births.	Seasonal Inocula-tions.	Per cent. Seasonal Inocula-tions to Births.
				Births.	Inocula-tions.			
November	1934	6	1	14	3			
„	1935	8	2					
December	1934	2	0	8	0			
„	1935	6	0					
January	1934	2	0	7	1			
„	1935	5	1					
February	1934	3	1	6	4			
„	1935	3	3					
November-February. Cold weather season						35	8	23
March	1934	5	2	6	3			
„	1935	1	1					
April	1934	3	1	4	1			
„	1935	1	0					
May	1934	1	1	5	2			
„	1935	4	1					
June	1934	3	3	8	6			
„	1935	5	3					
March-June. Dry hot weather season						23	12	52
July	1934	5	4	12	8			
„	1935	7	4					
August	1934	5	2	7	4			
„	1935	2	2					
September	1934	9	5	17	8			
„	1935	8	3					
October	1934	1	1	6	2			
„	1935	5	1					
July-October. The " rains "						42	22	52

early inoculation followed by a prolonged incubation period rather than a more recent inoculation with the usually short incubation period. It has thus been possible to determine those periods of the year during which malaria may be contracted in the Bengal Duars. But though this general information is of value, for one cannot afford to disregard any infective period, it has to be remembered that a period with a low inoculation rate is of less importance than one during which the inoculation rate is high.

Unfortunately when the number of records for any month or other period reveals a comparatively low incidence rate, one cannot conclude that there has been in that period a *de facto* low rate of inoculation ; because there is the possibility that inoculation has been followed by a long incubation. On the other hand, a proved high rate of inoculation is unequivocal, and active prophylaxis may be fairly based upon it.

The records here analysed, being comparatively few in number, have therefore been collated to show the number of births and the number of infants in which inoculation has been proved to have occurred not more than 60 days after birth.* The figures are arranged in three groups corresponding to the three chief seasons of the year (Table III).

Table II shows the time elapsing before certain selected children born in a particular month got malaria.† These children have been selected as giving precise and certain information, *i.e.*, they are for the most part children who developed malaria within a period of 4 to 6 weeks of birth.

It will be seen from this table that while a considerable proportion of the children born in each of the three main seasons of the year get infected within 60 days, there appears to be less chance of this happening during the cold weather, though this, of course, as stated above, may be due to a greater frequency of prolonged incubation at this season.

If now one collates the figures relative to births in any month and the grand total number of days elapsing before fever occurred in all the children born in any month one gets the result shown in Table IV (p. 250).

This table indicates the considerable chance a child born in any season has of getting malaria within a short time (those born in the rainy season having the greatest chance), yet as regards this chance the seasons do not differ from one another sufficiently to warrant a conclusion that a prolonged incubation period is a phenomenon particularly associated with any season.

Apart from these actual proofs of the periods of infection, there are in the Bengal Duars other indications that inoculation to a considerable extent occurs during all months of the year and that, while some cases of prolonged incubation may be associated with any season, they are more likely to occur during the cold weather.

*Arbitrarily taking those developing malaria within 70 days of birth.
†In this schedule some children born in 1936 have also been included.

TABLE IV.

Cold Weather Season	No. of Children	Total Days	Hot Weather Season	No. of Children	Total Days	Rainy Season	No. of Children	Tot Da
November, 1934	6	671	March, 1934	5	544	July, 1934	5	32
„ 1935	8	843	„ 1935	1	47	„ 1935	7	53
December, 1934	2	259	April, 1934	3	272	August, 1934	5	38
„ 1935	6	639	„ 1935	1	85	„ 1935	2	6
January, 1934	2	235	May, 1934	1	66	September, 1934	9	63
„ 1935	5	595	„ 1935	4	502	„ 1935	8	79
February, 1934	3	355	June, 1934	3	196	October, 1934	1	5
„ 1935	3	138	„ 1935	5	388	„ 1935	5	61
	35	3,735		23	2,100		42	3,40
	Average 107 Days			Average 91 days			Average 81 days	

CONCLUSION.

The object of this paper has been mainly to point out an economical method of ascertaining the periods during which malaria is inoculated by the mosquito into man ; and, thus to indicate if and when relaxation of prophylaxis may be allowed.

Although the data taken from the North Bengal Duars for the exemplification of the proposed method have been very insufficient for a complete elucidation of the local problem, it is interesting to note that inoculation by the mosquito in this region takes place certainly in the months of September, October, November and April and at least in the alternate months of the rest of the year, possibly in every month. The chances of inoculation within 60 days of birth are shown to be considerable even during the cold weather when the chances of infection are lowest. Further, there is little evidence to suggest that any particular season is associated with an unusually high inoculation rate or unusually long incubation periods, though as regards long incubation periods there seems to be some indication that these are most likely to occur in the cold weather.

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EPIDEMIC STOMATITIS IN THE NORTHERN SOLOMONS CAUSING RAPID DEATH.

BY

J. L. MEAGHER, M.B., B.S., L.M.

Medical Adviser, Marist Mission, North Solomon Vicariate).

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During September, 1935, there occurred an outbreak of stomatitis causing rapid death among the natives of a small island near Buka Passage, Territory of New Guinea. The disease, dramatic in its evolution, mainly affected the upper respiratory and alimentary tracts. Singular features were : The restriction of the outbreak to a limited geographical area, the appalling suddenness of some of the deaths, the extraordinarily high mortality, the absence of experience previously among natives of the locality of a sickness of this description, and the vivid clinical picture.

The island of Matsuhan, distant perhaps 6 miles from Buka Passage, was the scene of the outbreak. The resident population at the time, consisted, according to the natives' accounts in from fifteen to eighteen all told, comprising several families occupying four houses in the village which was near the beach. The occupation of the islanders is fishing, and they trade fish for taros, their staple food. The islanders are affiliated with the inhabitants of the neighbouring, much larger island of Fatsigern.

During the outbreak six of the inhabitants of Matsuhan died. The facts, according to the testimony of the *Kukurai* (Paramount Chief) of Fatsigern are : On Sunday, 15th September, a healthy male child aged 5 months, whose diet was breast milk and chewed taro, became ill at 6 p.m. It died at 8 a.m. on Monday. There had not been either headache or stomach ache. The child vomited often and cried with pain in the throat. The tongue was red. Other cases occurred on the following Thursday, the 19th of the month. The symptoms were, sore mouth, pain in the throat (occurring in some of the patients on the morning after the day of onset of the sickness) ; in some of the cases, vomiting— those who vomited died—dysphagia and slight cough. A premonitory symptom experienced by some was extreme malaise or weakness.

The sickness was entirely new in the experience of the natives. Red tongue was present in all the cases. None of the food taken by the Matsuhan Island natives at the time of the outbreak had been under suspicion by them. Upon being closely questioned on this point, the natives replied that they were poor people (the period was one of food shortage) and that taro was their only food. They denied that they had eaten fish immediately prior to the outbreak. There was not, in any of the cases, a history of belly ache. Nothing was said as to fever. Of those who fell ill with the characteristic symptoms, on Thursday, one young girl died the same night ; an adolescent girl died before dawn of the next day. One young boy died on Friday and an old man on Saturday night. There were no cases among the Fatsigern residents. On Friday morning, the 20th, the Matsuhan people migrated to Fatsigern. At the request of the *Kukurai* of Fatsigern, the writer visited that island on Sunday afternoon, the 22nd of September. The first patient seen was a woman aged about 45. She was dazed, lethargic and weak, with a staring expression and a slight cough. Her illness had begun on the 19th. The deep nervous reflexes were normal in type but gave sluggish response in the lower limbs. The tongue was furred with a biscuit-coloured fur. The fauces were injected. The temperature was 98·4° F. The heart sounds were distinct but subdued. The cardiac boundaries were within normal limits. The cardiac rate was 70 ; and the blood pressure, systolic 110, diastolic 80. Examination of the lungs and abdomen did not reveal any abnormality. This woman died during the night.

The second case was a strongly-built man of 35, who was walking about. He was dull in appearance, with little to say. The most striking clinical feature in his case was the carmine colour of the tongue ; and a purple shade on swollen lips, and within the mouth on the gums. The fauces, as in the previous case, were injected, but more markedly. An irregular, white, thrush-like fur was concentrated near the tip of the tongue, and also on the lips and at the sides of the mouth and on the gums. The tongue, at each side, bore depressions in the shape of facets, about 3 mm. square. The dorsum was glazed, with prominent, coarse papillae, appearing dirty white in a red field. The tongue was fissured longitudinally and transversely. The breath was without odour, and the contrast

between the red colour of the tongue and the mouth and the normal pink tinge of the tongues of the kanakas of Fatsigern was most marked. The tongue, on being protruded, deviated slightly to the left. Slight cough was present, but the lung findings were normal. Cardiac rate 90. Blood pressure, 140/110. The knee reflexes were normal in type but sluggish.

The third case was that of a boy of 15. He had been, like the others, taken ill on Thursday. He was thin and nervous, but lively enough and bright-eyed in contrast with the patients already noted. His tongue was red and glazed, but not fissured ; quite smooth, in fact. It exhibited papillae finer and more numerous than those on the tongue of the second case and more regularly disposed : the tongue protruded in normal fashion. The gums and buccal surfaces also were red. The cardiac boundaries were normal. Temperature 98·8° F. Pulse rate 114.

The fourth case was that of the sister of the second case, a woman aged about 35. She did not look very sick, though sluggish-looking and with eyes watering slightly. Cough was present. She was also taken ill on Thursday, but seemed to have recovered. The cardiac sounds were poor. There was no evidence of any pathological process in the lungs. The tongue was coated with a whitish fur.

Purgatives and aspirin were administered to those who were ill, and a sodium chloride mouth wash to the Matsuhan islanders. Next day Fatsigern was visited by the Government Medical Assistant at Buka Passage (Mr. J. LONG), and the village on Matsuhan was burned. There were no further deaths.

On the 25th of the month, a woman of 25, a native of Fatsigern, but resident at the Mission station, Tarlena, with her husband, presented herself for treatment for aphthous stomatitis. She considered she had contracted the same disease as the Matsuhan people : She had visited the island, it was said, some time before the outbreak ; according to another account, she visited the island on Thursday, 19th September, the day of the main outbreak. She fell ill on Friday the 20th, and had vomited. Clinical notes of her case, made at the time, are :—" She now has swollen, purplish, cracked lips, and a tongue closely resembling those seen at Fatsigern. The tongue is red-raw. It is covered, or rather strewn with, thirty whitish papillae, fifteen on each side of the mid-line, rather fine and irregularly-placed. At the junction of the hard and soft palates, there is a peppering of pin-point erythematous spots which coalesce toward the uvula and finally form a uniform deep-red surface. Tonsils and pharyngeal wall are of this colour. The sides of the tongue are indented, and on the pursed, dry, cracked, upper lip, on the lingual margin, are some small patches, of white, thrush-like material which could with difficulty be separated from the mucous surface by brushing with cotton-wool. No headache at onset. No cough. Eyes seem dull. Lungs : nothing abnormal. Heart : $\frac{1}{2}$ cm. right cardiac dulness. First sound at mitral area poor. Enlarged, firm spleen, reaching to umbilicus. Temperature : 97·8° F. Pulse rate : 69. The case was treated

with glycerine boracic acid applications to the mouth which it was possible to discontinue after 5 days.

On 3rd October the fourth case came to Tarlena for treatment of the condition. At the island, 11 days before, she had shown symptoms of coryza and was not very ill. Now she presented a pitiable appearance. A purulent conjunctivitis was present, matting the eyelashes. The lips were purplish and swollen, with cracks at the corners of the mouth. Attempts to examine the throat produced extreme salivation. A startlingly red, extremely tender tongue was glazed over almost the whole of the dorsum but bore at its sides a rather tenacious dirty-white fur. The dorsum was fissured longitudinally in two places and markedly transversely also at one level. The lingual aspects of the buccal mucous membrane bore lines of fur similar to that upon the tongue, and a similar fur coated the inner surfaces of the lips. The lateral aspects of the tongue bore facet-shaped indentations. Crusts appeared at the nostrils. Treatment consisted in mouth washes of a solution containing liq. arsenicalis minims ii to the half-ounce, and in glycerine boracic applications. Solutions of boracic acid, and drops of 10 per cent. argyrol were used for the eyes. She remained under treatment for one week, and received two injections of novarsenobillon (0·2 grammes intravenously and 0·2 grammes intramuscularly), during that period. She left on 11th October, with the condition of the mouth approximating to normal. A scraping from the tongue, stained with methylene blue, showed large numbers of Gram-positive bacilli morphologically similar to Hoffmann's cocco-bacillus in short chains, and large numbers of fusiform bacilli associated in great profusion with epithelial scales.

THE BREEDING OF *ANOPHELES COSTALIS* IN SEA-WATER, IN MAURITIUS.

BY

S. GEBERT.

Entomologist, Medical and Health Department. Mauritius.

Years back, I came across *Anopheles costalis* larvae, at the water's edge in the south-east of the Island, along the road from Mahébourg to Pointe d'Esny, in the fish-breeding enclosure known as " Barachois Rochecouste." Its water, in that particular part, is in direct communication with the sea and is strongly brackish.

More recently, I encountered *A. costalis* larvae in a small pool on the Pointe-aux-Sables beach, only a few feet from the sea, at the mouth of " Mare Samson." (Fig. 1.)

To the north of Port-Louis, on the southern shore of the Roche-Bois coast, a drain, known as Drain Marie, flows into a small creek which is itself part of the larger one known as La Mer Rouge, into which flows " Terre Rouge " River. Among the mangroves, at the mouth of the drain, lie several small pools of strongly brackish water in which *A. costalis* breeds, year in and year

out. At new moon and at full moon tides, during the flow, these crevices become covered and the larvae are carried a little further inland and, when the tide becomes low again, the larvae find themselves in pools containing a large proportion of sea-water. Thus, during the 4 or 5 days over which lasts the period of high tides, *A. costalis* breeds in water having a high salt content. (Fig. 2.)

After having studied this breeding ground for several months, and having made a few analyses of the water for chloride percentage, I came to the conclusion that it would be possible for *A. costalis* to live in pure sea-water and decided to verify the fact by means of breeding experiments. The analysis of the water sampled from the pools, just after the ebb, showed the following average amounts of chlorides reckoned as NaCl : during low-tide periods : 3·86 grammes per litre ; during high-tide periods : 10·27 grammes per litre.

The first experiment was started with first instar larvae found in a pool of water which showed, on analysis, 12·27 grammes NaCl per litre. Eighteen larvae were taken to the laboratory and were placed in sea-water, from Pointe-aux-Sables, containing 24·55 grammes NaCl per litre. During the first 5 days, six larvae died. Three days after, four more were dead. The remaining larvae, eight in number, grew normally and pupated from the 18th to the 20th day. After a pupal stage lasting 4 days, perfect imagoes emerged. In carrying out the breeding, the receptacle used was a finger bowl. No fresh water was added to make up for evaporation and, by the time the adults emerged, the water had concentrated down to about half its original volume. Analysis showed the high figure of 46·77 grammes NaCl per litre, that is nearly twice the strength of ordinary sea-water. As it was interesting to ascertain whether the concentrated water could still further breed *A. costalis*, I tried two other experiments with first instar larvae. These, however, gave negative results. The larvae which were taken from pools, containing respectively 3·21 and 4·36 grammes of NaCl per litre, died within 3 days.

Now, the above experiment was started with first instar larvae. As it might have been argued that, if young larvae could adapt themselves to sea-water, it was no proof that the eggs would hatch out in it, so I started another experiment with ova. After various unsuccessful trips to the breeding ground, I was fortunate in coming across a few newly-laid ova. Eight were found and taken to the laboratory and were placed in sea-water as before. Three days later seven ova hatched out. Four larvae died before reaching the second stage. The others grew normally and, 16 days after their birth, pupated and eventually normal-sized adults emerged.

In the absence, at that time, of a field station on the coast, the breedings had to be carried out indoors, in the laboratory which was situated 12 miles away from the breeding-ground and the larvae suffered during transit. In the laboratory they were deprived of the action of direct sunlight which is so beneficial to the development of *A. costalis*. Moreover, they were suddenly taken to an altitude of 1,000 feet and made to live in a climate which was not

theirs. Further, in the laboratory, they were fed artificially and were deprived of the numerous particles of organic dust which settle on the water in the open, and also of the microscopic and other algae found in their natural element, all of which serve as food to them. It is therefore remarkable that, under such adverse conditions, so many reached the adult stage.

FIG. 1.

Breeding pool at Pointe-aux-Sables beach.

FIG. 2.

Breeding ground at mouth of "Drain-Marie" with the creek known as Le Mer Rouge in the background.

These experiments show the remarkable adaptability of *A. costalis* to changes of environment and the possibility for it to live not only in ordinary sea-water, but also, perhaps occasionally, in the more concentrated sea-water in salt pans.

TROPICAL ULCER.

RESULTS OF EXPERIMENTAL INOCULATION IN HEDGEHOGS.

BY

E. C. SMITH, M.D.*

From the Medical Research Institute, Lagos, Nigeria.

STRONG and SHATTUCK *et al.*† described the production of lesions in monkeys following upon the inoculation of material obtained from tropical ulcers. In these experiments however, the skin at the site of inoculation had been previously bruised or otherwise damaged. With this exception no successful results in animals have been recorded. In Lagos, experimental inoculations have been made with the material from tropical ulcers into laboratory animals including monkeys, rabbits, guineapigs, white mice and white rats with entirely negative results. Either the inoculum has become absorbed, or an abscess has developed in which no spirochaetes or fusiform bacilli could be demonstrated.

In view of these findings, the results about to be described are of interest. The hedgehogs used in the experiments were obtained from Kano, Northern Nigeria. The inoculum consisted of material scraped from the surface, previously cleaned with saline, of tropical ulcers. The scrapings were emulsified in broth and examined for the presence of spirochaetes by the dark ground method, and for the presence of fusiform bacilli, in smears stained by Gram and by carbol fuchsin. ·

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†STRONG, R. P., SHATTUCK, G. C., BEQUAERT, J. C., WHEELER, R. E. (1926).   *Medical Report of the Hamilton Rice Seventh Expedition to the Amazon.*   p. 29.   Cambridge. U.S.A.: Harvard University Press.

Intracutaneous Inoculations.

The emulsion was inoculated in 0·5 c.c. amounts intracutaneously into the skin (shaved and cleaned with alcohol) of the abdominal wall. Fourteen animals were used. The abdominal skin of the hedgehog is very delicate and necessitates the use of fine needles for successful intracutaneous inoculation. Anaesthesia is necessary to induce the animals to relax completely.

In all the animals inoculated a well-formed bleb was present by the 2nd day and the surrounding skin was inflamed and indurated. Ulceration occurred from the 3rd to the 5th day. The ulcers varied in size from 1 to 3 cm. in diameter and were oval or circular in shape with hard, slightly raised edges. In the early stage the floor of the ulcers was covered with a tenacious grey-green foul smelling slough. Partial or complete separation of the slough occurred from the 5th to the 7th day, disclosing a purulent base composed of granulation tissue and necrotic material. In most of the animals inoculated the ulcers had healed by the 11th day but in two instances the lesions remained active until the 23rd and 25th days respectively. In three instances death occurred on or about the 7th day of infection. Death was presumably due to toxaemia since no gross lesions were found at autopsy and cultures of the heart's blood and of the spleen were negative.

Subcutaneous Inoculations.

Seven animals were used and were inoculated subcutaneously with amounts varying from 0·25 to 0·5 of the emulsified material. The inoculations were made in the region of the abdomen, forehead and groin. Well marked abscess formation with surrounding oedema was present on the 4th day in all of the animals inoculated. The lesions commenced to break down and to discharge a thin brownish fluid by the 5th to the 6th day. In three instances large irregular ulcerated areas formed and persisted for periods varying from 15 to 30 days. One hedgehog which was inoculated with 0·25 c.c. of the material in the skin of the forehead died on the 9th day. An extensive ulcerated area was present. The floor of the ulcer was formed by a necrotic foul-smelling green slough. When the slough was removed muscle tissue was exposed. The postmortem findings, except for generalised congestion, were negative.

Passage Experiments.

A second passage was made in six instances, a third in five, a fourth in two, a fifth in two and a sixth in one instance. For these experiments the material was scraped from the surface of the experimental ulcers, emulsified in broth and inoculated intracutaneously in amounts varying from 0·1 to 0·5 c.c. Ulcerated lesions were produced which resembled those already described. The passage experiments were unfortunately interrupted owing to lack of hedgehogs.
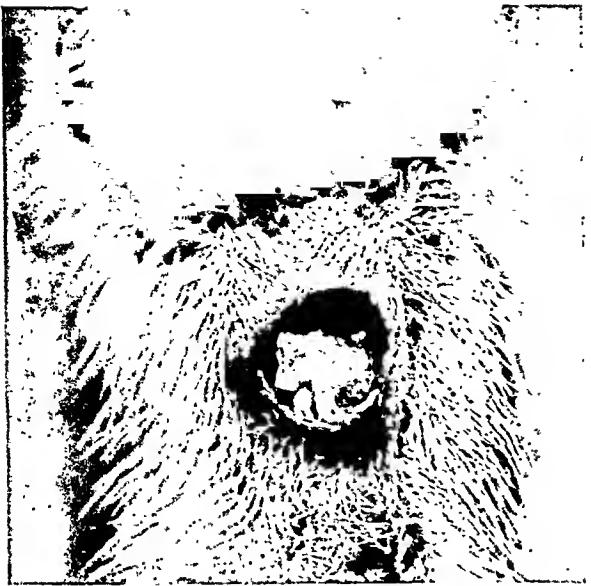
FIG. 1.—Type of experimental ulcer produced in hedgehogs. Six days' duration.
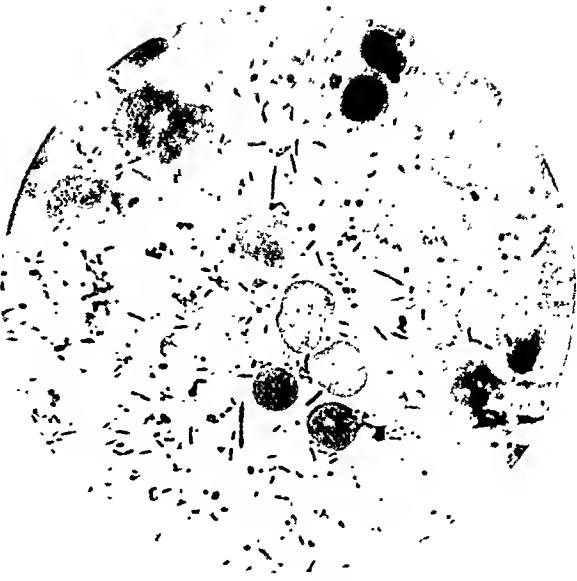
FIG. 2.—Smear from an experimental ulcer showing presence of spirochaetes, fusiform bacilli and mixed infection. ×835.
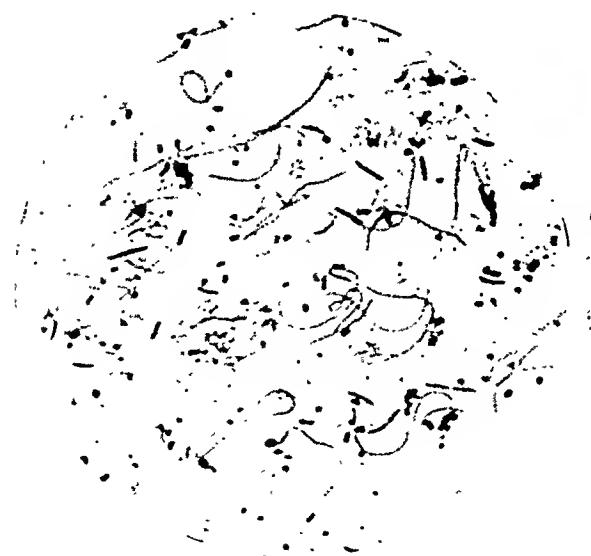
FIG. 3.—Smear from an experimental ulcer. Large numbers of a long, slender type of fusiform bacillus present. ×835.
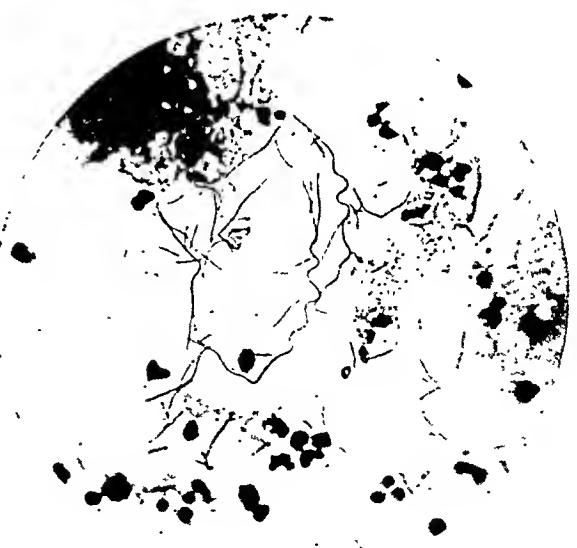
FIG. 4.—Smear from an experimental ulcer. Fusiform bacilli of unusual length present. ×925.

Microphotographs by Mr. J. E. Knight.
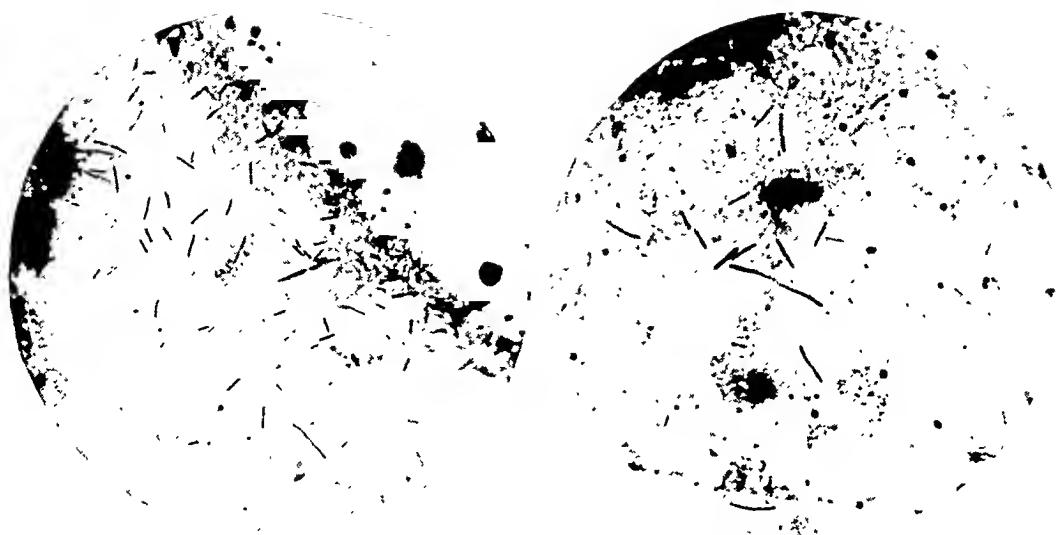
FIGS. 5 & 6.—Smears from experimental ulcers showing fusiform bacilli in the process of division. ×790. ×835.

FIG. 7.—Smear from an experimental ulcer showing an unusual type of fusiform bacillus. ×925.
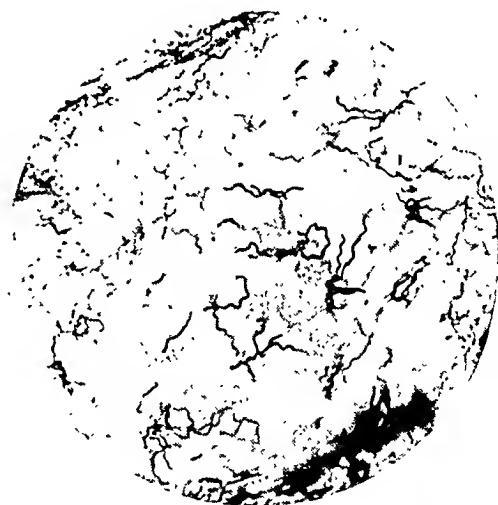
FIG. 8.—Scraping from the base of an experimental ulcer which had almost healed (20 days' duration). Numerous spirochaetes present. ×925.
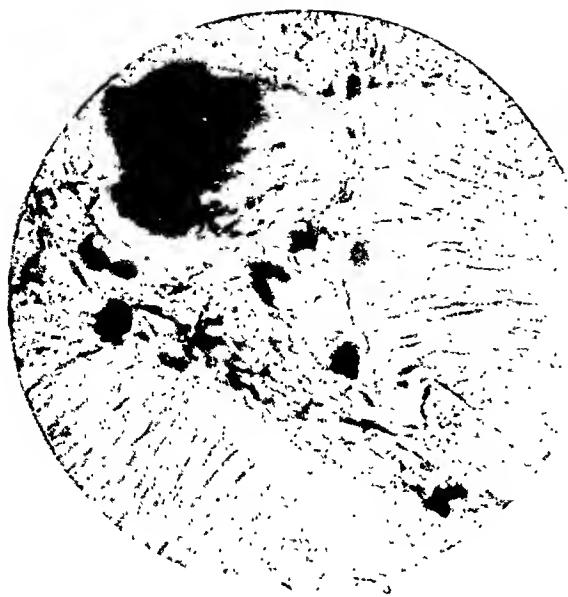
FIG. 9.—Section of the base of an experimental ulcer. Invasion of muscle fibres by fusiform bacilli. ×835.
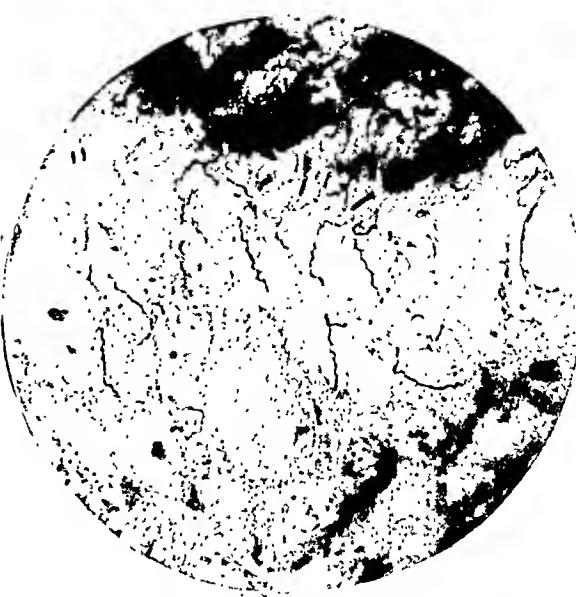
FIG. 10.—Section of the edge of an experimental ulcer. Spirochaetes of the *Treponema vincenti* type present in the superficial epithelium. ×925.
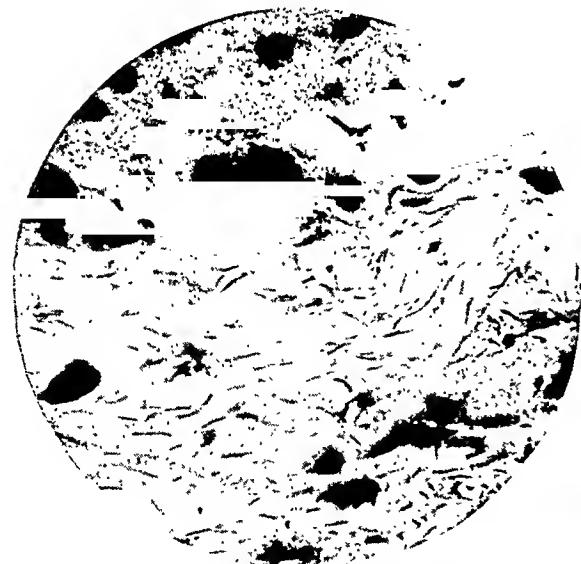
FIG. 11.—Section from the edge of an experimental ulcer. Invasion of the corium by fusiform bacilli. ×835.
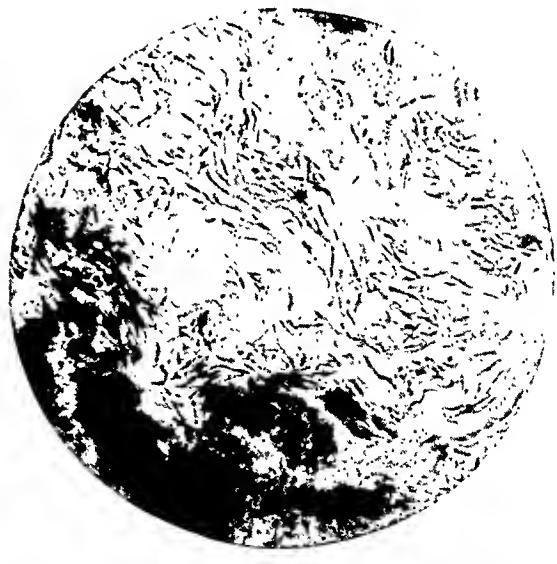
FIG. 12.—Section from the base of an experimental ulcer. Dense masses of fusiform bacilli and necrosis of muscle fibres. ×835.

Microscopic Examination.

Fresh preparations of the discharge from the lesions were made daily and examined by the dark ground. Smears were also made and stained by Gram's method and by Giemsa.

Spirochaetes of the *Treponema vincenti* type were demonstrated in all of the preparations and persisted throughout the duration of the lesions. They varied greatly in number.

In the stained smears, fusiform bacilli of various types were present. An extremely slender, long, motile organism, presumably a variety of fusiform bacillus, was present in excess in some of the smears examined. When healing commenced the spirochaetes and fusiform bacilli rapidly disappeared from the lesions but on two occasions when preparations from a clean, almost healed ulcer of approximately 25 days duration were examined with the dark ground, spirochaetes were present in large numbers whilst fusiform bacilli were extremely scanty. In many of the stained smears examined, fusiform bacilli were noted within polymorphonuclear leucocytes. Fusiform bacilli with central swellings were also observed as well as others which appeared to be about to undergo transverse fission.

Other organisms were also present including Gram-positive cocci and Gram-negative bacilli.

A short Gram-negative vibrio was present in some of the preparations.

Morbid Histology.

Biopsies were taken from two of the ulcerated lesions when well established (6 to 10 days). The piece of tissue removed included the edge and a portion of the base of the ulcer. Sections were prepared and stained by haematoxylin and eosin, by Giemsa and by the Warthin Starry method for the demonstration of spirochaetes. Microscopically there was found to be a well marked inflammatory reaction and necrosis of the tissues in the region of the ulcer. The reaction was widespread and involved the deep seated muscle fibres which were in places separated by a dense infiltration with polymorph cells. In the sections stained by the Warthin Starry method numerous spirochaetes were found to be present in the epithelium forming the edge of the ulcer and in the superficial necrosed tissue of the base. The fusiform bacilli were best seen in the sections stained by Giemsa. They were present in masses in the base of the ulcers, and isolated fusiform bacilli or small groups of this organism were present in the muscle fibres situated deep to the necrotic tissues.

At the edge of the ulcerated areas the fusiform bacilli had widely infiltrated the corium but none were seen in the epithelial layer.

A mixed infection, consisting mainly of cocci, was present on the surface of the ulcers.

SUMMARY.

Material from tropical ulcers, inoculated into hedgehogs has been found to give rise to lesions in which spirochaetes and fusiform bacilli, similar to the types found in tropical ulcer, have been demonstrated to be present throughout the duration of the experimental lesions.

Other organisms were also present.

Sixteen passage experiments have been made.

CONCLUSION.

Material from tropical ulcers, when suitably inoculated, is pathogenic for Nigerian hedgehogs, and spirochaetes and fusiform bacilli are present in the experimental lesions produced.

TROPICAL TYPHUS IN SINGAPORE.

BY

C. SUBRAHMANYAM, L.M.S. (SINGAPORE).*

From the Department of Pathology, Singapore.

ANIGSTEIN (1933) in his account of tropical typhus in Malaya noted that though the W form of the disease was urban in its distribution it had not been observed in the largest towns (Singapore, Penang, Malacca), an anomaly which awaited explanation. The cases to be described in this paper will show that the disease actually does occur in endemic form in Singapore.

The records of the General Hospital and the Tan Tock Seng Hospital, Singapore, show that one case of tropical typhus was reported by the General Hospital in 1932 while each hospital reported a case in 1933. In 1934 there were five cases in which the Weil-Felix reaction was carried out by the writer. The details of these are shown in the table on pages 264 and 265.

This result encouraged the writer in 1935 to carry out the Weil-Felix test in all sera sent to the laboratory for Widal tests. The result has been that of 1,209 bloods received forty-one were recognised as having belonged to cases of tropical typhus (W and K forms). It is clear from the histories of the 1934 and 1935 cases that with the exception of four (Nos. 5, 18, 28 and 45) all contracted the disease in Singapore itself and that the virus (W and K forms) exists in all parts of the town. Furthermore, it is evident that tropical typhus is not a new disease in Singapore and that the increase in the number of cases in 1935 is due to the general application of the Weil-Felix test and the fact that the medical officers in the hospitals have been on the look-out for the disease. Since the description given by FLETCHER and LESSLAR (1924) the number of cases diagnosed as " pyrexia of unknown origin " in Malaya have become progressively fewer but the difficulty of diagnosing tropical typhus without the help of the Weil-Felix reaction is well illustrated by the provisional diagnosis of cases in the series described here, namely enteric fever (several cases), myelitis, seven-day fever and " pyrexia of unknown origin."

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TABLE.

Nationality.	Sex.	Occupation.	Day of illness.	Weil-Felix titre. XI9 "O"	XK "O"	Widal reaction.	Total leucocytes.	Rash.	Remarks (R = recovered).
1934.									
1. Indian (Bengalee)	M.	Hawker	15 / 24	500 / 1,000	25 / 125	—	13th day 10,400	+	R. Severe form.
2. Indian (Malabari)	M.	Coolie	15 / 24	500 / 500	—	—	12th day 6,400	—	R. Severe form.
3. Indian (Sikh)	M.	Watchman	17 / 24	125 / 250	—	—	14th day 6,800	—	R. Mild form.
4. Chinese	M.	Nil	19 / 26 / 36	—	50 / 50 / 500	—	18th day 5,625	+	R. Severe form.
5. European	M.	Director	11 / 23	—	50 / 50 / 500	TABC "H" 1,000	—		R. Notes not available.
1935.									
6. Chinese	M.	School-boy	12	250	—	—	7th day 6,700	+	R. Mild form.
7. Indian (Tamil)	M.	Sweeper	9	1,000	25	—	8th day 4,400	—	R. Severe form.
8. Indian (Bengalee)	M.	Dhoby	13	—	250	—	6th day 6,100	—	R. Severe.
9. Indian (Tamil)	M.	Coolie No. 1287 C. Aerodrome	7	500	—	T "H" 25	—	—	R. Mild form.
10. Chinese	M.	Coolie	7 / 10	50 / 500	—	—	6th day 4,700	+	R. Fairly severe.
11. Indian (Tamil)	M.	Nil	11 / 19	250 / 25	—	—	13th day 5,800	—	R. Mild form.
12. Indian (Tamil)	M.	Coolie	7 / 14	1,000 / 250	—	—	6th day 9,400	—	R. Mild form.
13. Indian (Tamil)	F.	Married	13	1,000	50	T "H" 50	—	—	R. Mild form.
14. Chinese	M.	Coolie	12	250	50	B "H" 50	6th day 5,800	—	R. Mild form.
15. Indian (Tamil)	M.	Coolie	15	—	250	—	16th day 5,700	—	R. Severe form.
16. Chinese	M.	Merchant	8	1,000	—	—	7th day 4,600	—	R. Severe form.
17. Indian (Tamil)	M.	Clerk	14 / 10	2,500 / 250	25 / 25	T "H" 25 / B "H" 25	13th day 9,500	—	R. Severe type.
18. Indian (Malabari)	M.	Coolie	14	1,000	—	—	6th day 5,500	+	R. Mild form.
19. Chinese		Barber	7	—	—	—	—	—	R. Severe form.
20. Chinese	M.	Clerk	14 / 10	1,000 / 250	25 / 25	—	6th day 7,100	—	R. Mild form.
21. Chinese	M.	Coolie	14	—	—	—	13th day 6,500	—	R. Severe form.
22. Indian (Tamil)	M.	R.A.F. coolie	18 / 13	25	500 / 500	—	11th day 6,200	—	R. Fairly severe.
23. Indian (Tamil)	M.	P.C. 1160	21 / 8 / 15	1,251 / 500	1,000 / 500 / 25 / 25	—	8th day 4,300	—	R. Benign Tertian parasite in blood film. Severe type.

No.	Race	Sex	Occupation				Inoculation	Leucocytes	W.-F.	Remarks
25.	Indian (Bengalee)	M.	Nil	24	500	50	—	—	—	R. Severe form.
26.	Indian (Bengalee)	M.	Jaga	13	2,500	25	—	4th day 14,300	—	R. Mild form.
27.	Indian (Malabari)	M.	Nil	7 13	25 25⅞	25 500	—	4th day 10,000	—	R. Mild form.
28.	British	M.	Mining engineer	7 15 32	1,000	50 125 50	TABC "H" 50	10th day 5,300	+	R. Fairly severe.
29.	Chinese	M.	Coolie	13	250	50	—	8th day 4,200	—	R. Mild form.
30.	Indian (Tamil)	M.	Coolie	9	250	50	—	8th day 4,100	—	R. Mild form.
31.	Indian (Tamil)	M.	Coolie	14 17 25	1,000	250 250	T "H" 25 T "H" 50	17th day 2,400	—	Died.
32.	Indian (Malabari)	M.	Coolie	9	125	50	—	—	+	R. Mild form.
33.	Indian (Tamil)	M.	Sweeper	17 14 18	2,500 5,000 10,000	50 25	—	12th day 10,200 17th day 10,500	—	R. Fairly severe.
34.	Chinese	M.	Merchant	11 18	25	125 125	—	9th day 6,900	—	R. Temp. typical, though Weil-Felix low, severe.
35.	Malay	F.	Married	13 21	50 1,000	25	T "H" 50	—	—	R. Fairly severe form.
36.	Sikh	M.	Money lender	8	250	25	TABC "H" 25	5th day 6,500	+	R. Severe.
37.	Indian (Tamil)	M.	Coolie	8 14 22	—	25 125 10,000 250 250	T "O" 25	7th day 6,500 16th day 7,500	—	R. Severe form.
38.	Chinese	M.	Hawker	15	—	125 50	—	12th day 7,500	—	R. Mild form.
39.	Indian (Tamil)	M.	Nil	20	—	250	—	—	—	R. Case sheet not available.
40.	Indian (Tamil)	M.	Nil	20	125	125 50	T "H" 50 B "H" 125	—	—	R. Mild form.
41.	Indian (Tamil)	M.	Coolie	17	500	25	T "H" 50 A "H" 125	—	—	R. Severe form.
42.	Indian (Tamil)	M.		30	250		B "H" 50	—	—	R. Case sheet not available.
43.	Indian (Tamil)	M.	Coolie	11 20	50 125	25	—	No date 11,620	—	R. Mild form.
44.	Chinese	M.	Coolie	8 20	—	25 125	—	9th day 10,500	—	R. Mild form.
45.	Indian (Bengalee)	M.	Gunner	4	—	125	TABC "H" 50	—	—	R. Mild form.
46.	Indian (Bengalee)	M.	Thamby	9	1,000	25	—	6th day 17,100	—	R. Mild form.

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The first case of the series was seen in July, 1934. The blood had been sent for Widal test but as the result was negative and as the clinical features of the case were suggestive of tropical typhus the Weil-Felix test was carried out with the same sample of blood. The serum agglutinated *B. proteus* X 19 " O " antigen in a dilution of 1 : 500 and *B. proteus* XK " O " antigen in a dilution of 1 : 25. This specimen of blood had been taken on the 15th day of the illness. On the 24th day a further test was carried out giving readings of 1 : 1,000 and 1 : 125 respectively. This case is mentioned to illustrate the importance of the routine application of the Weil-Felix test.

Of the forty-six cases diagnosed between July, 1934 and December, 1935, thirty-seven were from the General Hospital and nine from the Tan Tock Seng Hospital. As only sera from the Government hospitals are sent to the laboratory it is clear that these cases must form but a fraction of the actual cases occurring in Singapore. In 1936 the practice of sending sera to the laboratory from the Mental Hospital was commenced with the result that a case of tropical typhus was diagnosed in February by the Weil-Felix reaction given by a serum submitted for a Widal test. The medical officer in charge remarked that this was the first case to be diagnosed at the Mental Hospital and furthermore was the first case of tropical typhus he had seen.

Both the urban (W) and rural (K) forms of tropical typhus are endemic in Singapore and as thirty cases of the former and sixteen of the latter occurred in the series it appears that the urban form is more common than the rural. This agrees with the findings of FLETCHER and LESSLAR (1925, 1926), LEWTHWAITE (1930) and ANIGSTEIN (1933). Both forms are prevalent both within and outside the town limits. From the point of view of the epidemiology of the disease it is of interest to note that two cases come from one house, two from the Naval Base coolie lines and two from the Royal Air Force coolie lines. Though all the patients were treated in the general wards of the hospitals there was no evidence of spread of the infection to other patients.

The forty-six cases showed the following racial distribution :—Tamils twenty-three, Bengalees six, Sikhs two, Chinese twelve, Europeans two, Malay one. It is worthy of note that ANIGSTEIN (1933) referred to the striking preponderance of the disease among the Tamils who actually form only 20 per cent. of the total population of Malaya.

As regards symptomatology there is nothing of outstanding importance to note. In eleven out of fifteen cases a moderate leucopenia (average 5,400) was noticed during the first week of illness. ANIGSTEIN (1933) noted leucopenia in only 30 per cent. of his cases. During the second week thirteen cases showed an increase in the count as compared with the above series (average 7,600), while two cases gave a leucocytosis of 14,300 and 10,000 respectively. The temperature charts of most of the cases showed fever of the continuous type, ending usually in rapid lysis. They resemble more closely the charts of European typhus and those given by FLETCHER and LESSLAR (1926) than those accompanying

the description by ANIGSTEIN (1933). Only one case (K form) of the forty-six proved fatal. There was no definite rise or fall in the monthly incidence, a feature which was noted also by ANIGSTEIN (1933). It may be accounted for by the very slight variations in climate experienced in Singapore.

Several strains of *B. proteus* have been isolated during the course of the investigation from urine, faeces and abscess pus. Some of these resemble *B. proteus* XK both in biochemical and antigenic (H and O) properties. It is not possible to give more details of these strains at present but they are mentioned
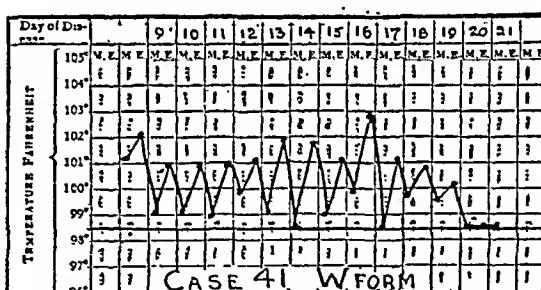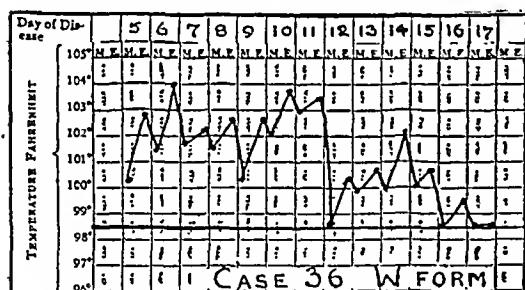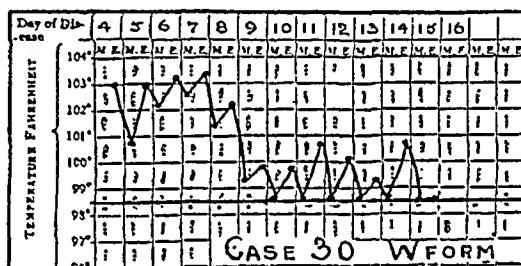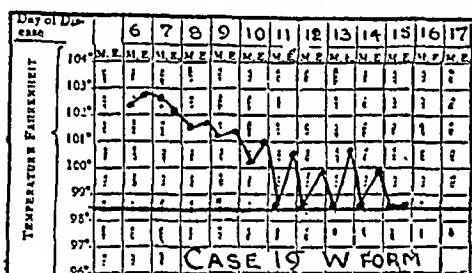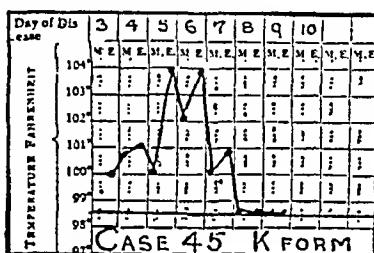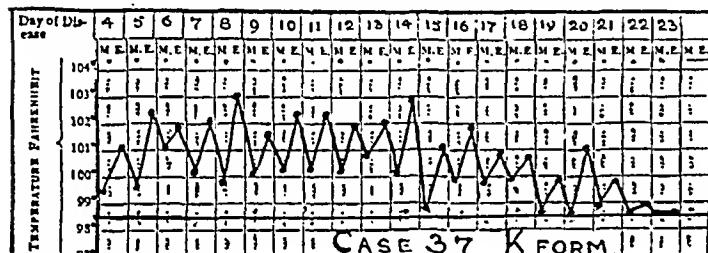
Temperature Charts of six cases.

to show that such strains occur in close connection with tropical typhus as already pointed out by FELIX (1933). The strains of *B. proteus* (OX 19 and OXK) used for the Weil-Felix reaction were kindly supplied by the Institute for Medical Research, Federated Malay States. Alcoholized suspensions of the strains, which contained the O factor only were used in the reaction. Our findings as regards the diagnostic titre (1 : 125 and over) agree with those of LEWTHWAITE (1930).

Tropical typhus is thus a disease which occurs in all parts of Singapore and it is evidently desirable from what has been noted by other observers that attention be directed towards the rat, the rat-flea, ticks, mites and lice from the point of the discovery of the reservoir and vectors of the infection. It is possible that the O antigens of the *proteus* X strains might be used for prophylactic inoculation but this is a question requiring investigation.

SUMMARY AND CONCLUSIONS.

1. Tropical typhus is shown to be endemic in Singapore.

2. The W form occurs more commonly than the K form.

3. The value of carrying out the Weil-Felix test on all bloods sent in for Widal test is noted.

4. The temperature charts of the cases were similar to those of cases of European typhus.

5. In the majority of cases there was a moderate leucopenia.

6. The close association of *B. proteus* X strains with the disease is pointed out.

7. The use of O antigens of the *proteus* X strains for prophylactic vaccination is suggested.

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CORRESPONDENCE.

THE MODE OF ONSET OF THE CEYLON MALARIA EPIDEMIC.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

May I be allowed to correct the following mis-statements in Colonel GILL's communication on the mode of onset of the Ceylon epidemic which was published in the TRANSACTIONS, Vol. XXX, No. 1, June, 1936 ?

On page 101 Colonel GILL says that in a paper read at a meeting of the Royal Society of Medicine (Section of Tropical Diseases) on 5th March, 1936, I concluded that the Ceylon malaria epidemic started gradually and was associated with a slow, steady increase of primary infections. As I was particularly careful not to use the word " slow " in my paper its insertion in this and other sentences of Colonel GILL's communication is incorrect. I said no more than that the onset seemed to me to be " gradual " and in replying to the discussion on my paper I made the remark that even to speak of the onset of the Ceylon epidemic as having been " gradual " might be misleading unless it were mentioned that the word was being used to mean only " proceeding step by step " without reference to speed.

On the same page Colonel GILL says that I made no reference to his hypothesis that the epidemic started suddenly by means of an " epidemic of relapses." If he had attended the meeting at which my paper was read he would have heard the following :—

"In concluding this paper I should like to make it clear that although what I have said seems to show that the mode of onset of the epidemic was different from that suggested by Colonel GILL in a previous discussion, I am not myself committed to one view rather than to the other. Colonel GILL, in the discussion referred to, advanced strong reasons for the view that the onset of the epidemic was sudden and explosive, the prime cause being an outburst of relapses. What I have done is to advance reasons for the view that the onset was gradual, that it consisted of an increase of primary cases rather than relapses, and that it did not become explosive until it had been increasing steadily for six weeks or more. I have stressed this view in order that workers may be in a position to study the problem from both aspects before arriving at a final conclusion with regard to it."

I am, etc.,

S. P. JAMES.

21, Harrington Road,
S.W.7
22nd July, 1936.
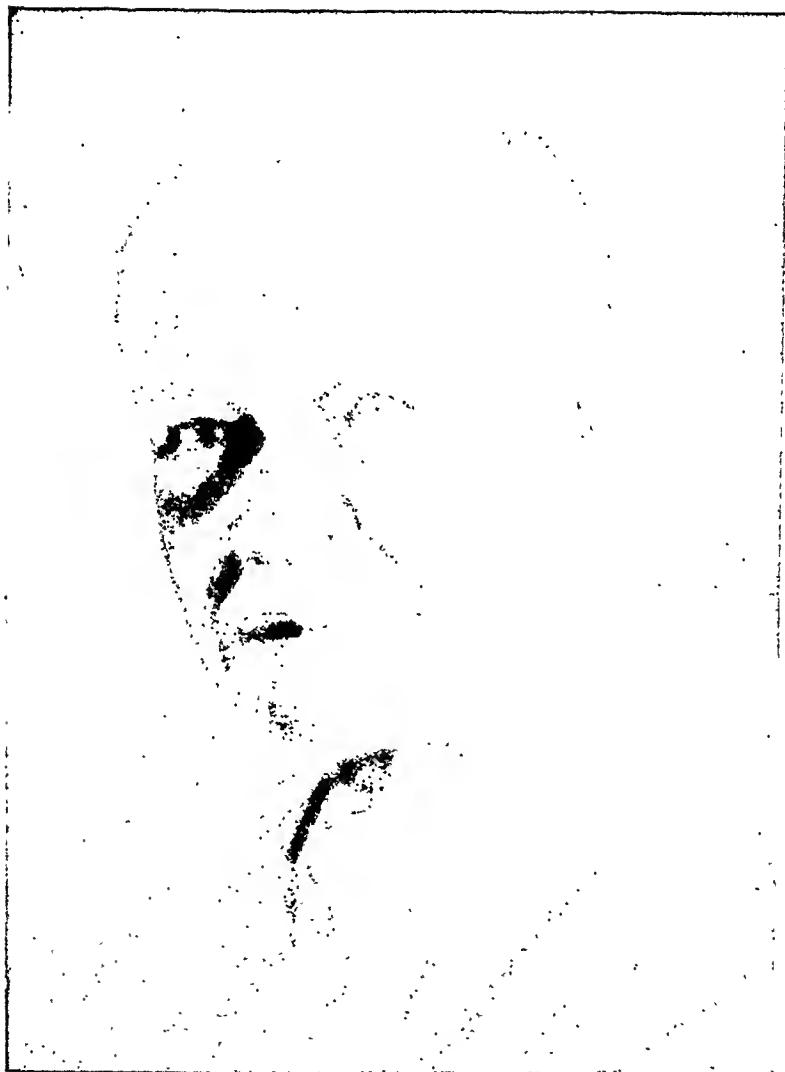
OBITUARY.

CHARLES NICOLLE.

1866—1936.

CHARLES NICOLLE, Director of the Pasteur Institute of Tunis and Honorary Fellow of The Royal Society of Tropical Medicine and Hygiene since 1911, died at the age of 70 on February 28th of this year. With his death our Society loses one of its most distinguished Honorary Fellows, and Medical Science one who has contributed materially to its advance in many directions during the past 30 or 40 years.

After early study and work in France, where he proved himself to be an investigator of outstanding promise, he was appointed in 1903 Director of the Pasteur Institute of Tunis, founded 3 years before. From very modest beginnings the Institute, under CHARLES NICOLLE's influence and direction, has risen to a position of world-wide importance and reputation. Its band of distinguished workers, headed by the illustrious Director, has carried out researches of fundamental importance to tropical medicine and hygiene. In this connection it is only necessary to mention the subjects of typhus fever, relapsing fever, infantile and canine kala-azar, from a list of many others, to bring to mind the name of CHARLES NICOLLE and the profound influence he has had on our present day knowledge of these and many other diseases. His work, particularly that on typhus fever, its transmission by the louse and infectivity to guineapigs and other animals was recognised by the award in 1928 of the Nobel Prize for Medicine, which, with the possible exception of his election to the Académie des Sciences in 1929, is perhaps the highest of the many honours which were bestowed upon him.

PUBLICATION OF MONOGRAPHS AS SUPPLEMENTS TO THE TRANSACTIONS.

The Council has decided to extend the scope of its publications by issuing from time to time Monographs on suitable subjects. These will be supplements to the TRANSACTIONS and will have the same form. They will be sent gratis to all Fellows of the Society. It has not yet been decided how often they will be published : much will depend on the suitability of the material submitted, but the publication of one Monograph a year would, at the present time, appear to be the probable extent of the issue.

Monograph No. 1 now in the press is by C. J. HACKETT, and will deal with the subject of Boomerang Legs and Yaws in Australian Aborigines, a preliminary account of which is given by him in this number of the TRANSACTIONS.

CORRIGENDA.

Vol. XXX, No. 1. June, 1936.

Dr. N. HAMILTON FAIRLEY on Tropical Sprue.

Page 14, line 29 : for 25·9 mg. per 100 c.c. read 259·8 mg. per 100 c.c.

Dr. JANET VAUGHAN in Discussion.

Page 56, line 41 : for " mannite " read " marmite "

Vol. XXIX, No. 6. April, 1936.

Prof. F. O'B. ELLISON on Malaria Epidemics and Sun Spots.

Page 662, line 36 : for 6/100 read 3/100

Page 663 : Table A (rainfall figures) should read as below.

		At Sun-spot Maximum.	Half Down.	At Sun-spot Minimum.	Half Up.
Wet	Colombo (not malarious)	95	80 (57)	101 (140)	103
Wet	Kurunegala (very malarious)	80	84	92	100 (140) (67)
Dry	Anuradhapura	54 (39)	49	54	58 (75)
Very wet	Ratnapura	152 (135)	150	165 (191)	170
Dry	Puttalam	42	40	47	53 (86) (32)
Dry	Mannar	36	39	33 (21)	44 (64)
Dry	Jaffna	50	48	46 (30)	54 (78)
	Badulla	75	65 (52)	76 (52)	89 (151)
Hill	Hakgala	90 (75)	90	105 (133)	107
Hill	N'Eliya	84 (68)	92	106 (128)	97
Hill	Kandy	78 (64)	87	95 (117)	86
Dry	Trincomalie	65	58	68 (35) (95)	57
Dry	Batticaloa	64	58	67 (35) (113)	65
Dry	Hambantota	39	40	44 (60)	40 (18)
Wet	Galle	84 (68)	95	117 (143)	102

TRANSACTIONS

OF THE

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.

Vol. XXX.   No. 3.

Proceedings of the **Opening Meeting of the 30th Session of the Society**
held at Manson House, 26, Portland Place, London, W.1, at 8.15 p.m.,

on Thursday, 15th October, 1936.

Sir ARTHUR BAGSHAWE, *C.M.G.*, M.B., D.P.H., *President*, in the Chair.

The President : Since the Society last met we have lost by death three
outstanding Fellows, and I think I should make a brief reference to them before
we come to our business for the evening.   They are two Honorary Fellows—

Sir HENRY WELLCOME and Sir ARNOLD THEILER—and the third is Dr. WILLIAM HARTIGAN, an Original Fellow of the Society who was less well known, probably, to our younger Fellows.

Sir HENRY WELLCOME's interest in tropical medicine is well known, but what is, I think, of special interest to us is the full and free assistance which we have always received from the Wellcome Bureau over a long series of years, and also the fact that Sir HENRY subscribed generously to our Manson House Fund.

Sir ARNOLD THEILER enjoyed a world-wide reputation for his researches in animal medicine, researches which he continued long after the accustomed retiring age, and nearly to the end of his life.

Dr. HARTIGAN was our first Treasurer, namely, from 1907 to 1909 ; he served on the Council till 1921 ; and since then he had been one of our Trustees.

PAPER.

RECENT OBSERVATIONS ON THE BIOLOGY OF THE TRYPANOSOMES OF MAN IN AFRICA.

BY

H. LYNDHURST DUKE.

I.—INTRODUCTION.

Left to myself I should never have presumed to attempt the task implicit in the title of this paper. Having recently retired from the Colonial Service after a 25 years' acquaintance with African trypanosomes, I considered that my obligations ceased with preparation of a series of papers embodying the final results of the work done in the Uganda Institute. The last two papers of this series are due to appear in the next number of *Parasitology*. It was represented to me, however, during the last few weeks, by a prominent member of this Society, that a discussion on the outstanding problems of sleeping sickness was long overdue, and that a paper setting forth the main results achieved by recent research might stimulate a useful interchange of ideas. So much by way of apology and explanation. If I shall have a great deal to say about my own work it is because I am one of the few in Africa who have been privileged to bring their studies to something approaching completion. Others, less fortunate or less irrepressible, have been from time to time interrupted or suppressed. Many of the results quoted in this paper are, as stated, actually still in the press. I have been fortunate in learning by letter a few weeks ago from Dr. VAN HOOF of some of the interesting results he and his colleagues have obtained at Léopold-ville, and which he is at present preparing for publication. Some of these I am able to mention here. Only recent research will be considered and the therapy of sleeping sickness will not be touched. The subject is so wide that I fear there will be some unintentional omissions. The success of this paper will be measured by the discussion it provokes, either immediately or later in the press ; and I look forward with interest and some trepidation to learning the views of those whose opinions have not been so voluminously expressed as my own. It is possible, too, that those interested in other blood parasites of man may be able from their knowledge of these organisms to throw some light on the principles governing the behaviour of all these protozoa.

The headings of the various sections indicate the line of approach towards a reasoned opinion on the main outstanding questions of this research, namely, the rôle of the big game in the spread of human trypanosomes in Africa and its complementary problem, the origin of *Trypanosoma rhodesiense*. It is not to be expected that all the inferences and theories put forward in this paper will prove acceptable to all those engaged in investigating these problems. There is, however, some consolation in the thought that the greater the divergence of opinion, the more useful the discussion.

II.—FACTORS THAT MAY INFLUENCE THE ADAPTATION OF MAN'S TRYPANOSOMES TO TSETSE.

A number of papers from Uganda have been published on this subject during the last few years, the findings of which will now be briefly summarized.

(a) Among the characters by which different strains of trypanosomes are distinguished from one another must, I believe, be included resistance to drugs, virulence, and transmissibility by tsetse. All or any of these characters, though fairly stable, may, quite independently of one another, change from time to time in the history of a strain. For example, when a trypanosome remains for a long time in a vertebrate, and especially when its presence causes a chronic and progressive disease in the host, the strain tends to lose its power of development in tsetse.

(b) The transmissibility of the *brucei* trypanosomes depends on their power to become established in the salivary glands of *Glossina*. My own work has shown that a strain may lose its power of invading the glands of the fly while still capable of developing in the intestine. Such a strain in nature would be just as incapable of spread as one that had lost all power of development in tsetse. A strain known to be non-transmissible by *G. palpalis* was equally incapable of development in *G. morsitans*. There is some support for these observations outside my own publications.

(c) I have found that *T. rhodesiense* is, as a rule, better adapted to development in tsetse than *T. gambiense*, though there are exceptions in both directions.

(d) I have also found in nature strains of *T. gambiense* that apparently cannot develop in tsetse. Presumably the loss of transmissibility had taken place in the host from which they were isolated. (DUKE, 1930a.)

VAN HOOF tells me that he has confirmed the existence in the Congo of similar strains in man, and that the transmissibility of strains of *T. gambiense* from this region is often very feeble.

(e) Another interesting point is that the *G. palpalis* of Lake Victoria is not such a good transmitter of the *brucei* group as *G. morsitans* from Tanganyika (DUKE, 1933a). LESTER (1934) has recently found in Nigeria that *G. tachinoides* is a better transmitter of man's trypanosomes than either *G. palpalis* or *G. submorsitans*, and some of CORSON's experiments suggest that *G. pallidipes* is a very efficient transmitter of this group of trypanosomes. The experiments performed in the Uganda Institute have thus been carried out with one of the worst transmitters among the common species of tsetse, a definite handicap to investigations which depended so largely on the use of tsetse.

(f) No difference in transmitting power was detected between an island and a mainland race of *G. palpalis* in Uganda, when exposed to identical conditions of infection. KLEINE and ECKARD (1913) carried out experiments which showed that the offspring of tsetse which had been fed for weeks upon blood rich in trypanosomes were not better transmitters than the descendants of flies fed on clean blood.

(g) As to temperature, years ago KINGHORN, YORKE and LLOYD (1913) in Rhodesia found that invasion of the salivary glands of *G. morsitans* did not take place below 75° F., whereas the alimentary canal became infected at considerably

lower temperatures. More recently TAYLOR (1932) found that by keeping *G. tachinoides* at 98° F. instead of at room temperature for the 4 days of infecting feeding, an enormous increase occurred in the number of infected flies. He ascribed this to the effect of the temperature on the trypanosomes in the fly ; but on repeating his experiments with *G. palpalis* I was led to conclude that the explanation of his results lay mainly in the effect of the higher temperature on the physiological processes of the fly itself (DUKE, 1933d). More recently again, YORKE at Liverpool and VAN HOOF in the Belgian Congo have failed to reproduce TAYLOR's results in experiments carried out with *G. palpalis*.

(*h*) Recently, in VAN HOOF's laboratory at Léopoldville, BRUTSAERT and HENRARD have succeeded in infecting animals with inoculations of test-tube cultures of *T. congolense*. Similar experiments with the *brucei* group failed. This is the first instance of a culture of an African pathogenic trypanosome proving infective on inoculation into a mammal.

(*i*) A remarkable and unexpected result obtained both in Uganda (DUKE, 1934) and in Nigeria by LESTER (1934) is that repeated cyclical passage of a strain through tsetse does not apparently affect the transmissibility, though on one occasion virulence was reduced.

(*j*) It has been found also that repeated cyclical passage of *T. brucei* or *T. rhodesiense* though *G. palpalis* does not change the main characters of the strain, and LESTER has reached the same conclusion with *T. gambiense* and *G. morsitans* in Nigeria (1934). He also showed that acquired resistance to human serum was unaffected by cyclical passage through tsetse. All these observations are important to a proper understanding of trypanosomiasis in human communities.

(*k*) Another important phenomenon is that on different days the trypanosomes in the blood of the mammal may differ considerably in their power of developing in the tsetse. This observation was first made by ROBERTSON (1912) in Uganda, and as far as my experience goes it is undoubtedly correct. It is very striking to find in a strain of readily transmissible trypanosomes that on certain days, sometimes for several days in succession, none of the flies fed on the animal develop infections, although trypanosomes are plentiful in the blood.

The recent work of BROOM, BROWN and HOARE (1936) on the electrical charge carried by trypanosomes may provide an explanation of this remarkable phenomenon. It was suggested by HOARE (1923) that the development in the test-tube and in fly are both manifestations of the same character, and the Belgian results with *T. congolense*, just referred to, confirm this conclusion. BROWN and his colleagues have found that the cultural forms of all the species of trypanosomes they have examined, both in the tube and in the insect, carry a negative charge. They have also found that positively charged trypanosomes are more sensitive to arsenical drugs than those bearing a negative charge.

It would be interesting to apply this electrical method to strains known to be non-transmissible by tsetse, and to see if the changes in transmissibility exhibited by successive phases in the endogenous cycle in the mammal correspond with changes in electrical charge. Such an investigation would be most instructive if carried out on a host such as the monkey or man in which the trypanosome produces a chronic infection lasting a long time. BROWN and his colleagues found that *T. evansi*, a species which is generally described as unable to develop in tsetse, may exhibit both negatively and positively charged trypanosomes in infections in guineapigs. But it remains to be seen whether no positively charged trypanosome can develop in test-tube or culture.

The results obtained by WALLACE and myself (DUKE, 1930a) with the red-cell adhesion test in the diagnosis of trypanosome infections in man and animals require reconsideration in the light of BROOM, BROWN and HOARE's observations. We found that adhesion of red-cells to trypanosomes occurred when a strain was exposed to the action, *in vitro*, of a homologous serum. The reaction was only pronounced in the presence of primate corpuscles, either of man or monkey. A certain amount of non-specific adhesion also occurred, but this never affected more than a small percentage of the trypanosomes present.

It is difficult to believe that our results, obtained in a great number of tests, were purely fortuitous and of no diagnostic significance, and it will be interesting to determine whether the presence of its homologous serum exerts any effect on the electrical charge carried by a trypanosome.

(*l*) There is some evidence that the species of mammalian host may influence the transmissibility of a trypanosome by tsetse. I have found that the guineapig apparently exerts an unfavourable influence in this respect, also that there is reason to believe that certain species of antelope in Uganda, for example, oribi and situtunga, are less suitable to the requirements of *T. rhodesiense* than the bushbuck and the reedbuck (DUKE, 1935a).

CORSON (1935 and 1936a) has recently described very striking evidence on this point obtained with a large species of reedbuck in Tanganyika. In contrast to the commoner species, this reedbuck on two occasions gave rise to an extraordinarily high percentage of gland infections among the clean flies fed upon it, in one experiment 64 per cent. It is not at present clear whether the antelope suffered from the trypanosome infection and whether the high infectivity was retained for a long period.

(*m*) It is not uncommon to find wild tsetse infected with more than one species of trypanosome, an observation that suggests that certain individual flies are particularly suited to the requirements of the developing flagellates. A remarkable instance of double infection is that recorded by JOHNSON and LLOYD (1923) who dissected 19,000 wild *G. morsitans* in Nigeria, and found seven infected in both gut and salivary glands, and of these seven flies, six were also infected with *T. congolense*. I found the same phenomenon in a *G. swynnertoni* in the Mwanza region of Tanganyika.

(*n*) No instance has been recorded of one species of tsetse being unable to transmit a trypanosome that is known to be transmissible by another species.

(*o*) TAYLOR believes that a strain is potentially transmissible cyclically by tsetse as long as it is capable of development in the proventriculus of the fly. VAN HOOF and HENRARD have recently investigated this point, but were unable to reach a decision whether the proventriculus plays an essential rôle in determining the infection of the salivary glands.

These observations on the transmissibility of trypanosomes by tsetse suggest certain reflections. In comparison with the successful adaptation shown by other species of trypanosome to their intermediate hosts, the *brucei* group and, indeed, all the tsetse-carried pathogenic trypanosomes, do not appear to have advanced very far towards perfection. The three groups of protozoa that live in man's blood, plasmodia, trypanosomes and leishmania, show different degrees of adjustment to their insect carriers. The plasmodia of malaria, in all probability for ages parasitic on man and dependent on mosquitoes, have attained a high degree of adjustment to both final and intermediate hosts. Certain prevailing species of mosquito are specially associated with the spread of these protozoa, but they can also develop in species that are seldom or never called upon in nature. Adaptation of the *brucei* trypanosomes to cyclical development in tsetse, even in the most favourable circumstances is, as we have seen, not very great. Least successful of all are the leishmania in their adaptation to full cyclical development in *Phlebotomus*. May not the degree of adaptation of these protozoa to the insect afford an index of the antiquity and, therefore, of the epidemiological significance of the association between them ?

III.—ARSENIC RESISTANCE.

We now pass to a brief consideration of arsenic resistance in trypanosomes, a subject which I approach with considerable diffidence. YORKE and his colleagues in Liverpool, by means of their *in vitro* method, have added much to the knowledge of the nature of acquired drug resistance in the *brucei* group. In the course of their researches they succeeded in demonstrating that cyclical passage through tsetse had no effect on an acquired increase in the drug resistance of a strain of *T. brucei*. In nature *T. brucei* and *T. rhodesiense* possess a degree of natural resistance to arsenic that precludes the use of arsenicals in the treatment of the diseases they produce. *T. gambiense* in its typical mild form is as a rule susceptible to arsenic in therapeutic doses. In recent years there have been found in Uganda (DUKE, 1930), in Nigeria (LESTER, 1933), and in the Belgian Congo, strains of *T. gambiense* that possess a considerable degree of resistance to arsenic. These strains have been found in areas where no treatment of natives has been employed. It is evident from the Liverpool work that the resistance of these strains could readily be enhanced and that faulty treatment

with arscnicals might bring about this result. In Nigeria, for cxample, where many varieties of strains occur, such a danger is very real.

With *T. gambiense* thc position is more complicated. This trypanosomc as we have seen is prone to lose its transmissibility by tsetse. It has proved very difficult experimentally to make *T. gambiense* resistant to arsenic. WALLACE and I in Uganda (DUKE, 1933c), and LESTER (1934) in Nigeria both succeeded with much trouble in producing a resistant strain, but in both cases it had in the process of preparation lost its power of devcloping in tsetse. VAN HOOF tells me that he also has succeeded with difficulty in making strains of *T. gambiense* resistant to arsenic. Their transmissibility at the end of the drug course was much reduced or completely lost, but revived somewhat when the. strain was inoculated into a fresh host. His experiments are not yet fully described, but it is evident that such a reduction of transmissibility in a strain in nature would render its survival extremely unlikely.

LESTER, in his 1934 Annual Report, again lays stress on the difficulty of experimentally making *T. gambiense* resistant to tryparsamide, and remarks " there is, as yet, no experimental proof that repeated ineffective treatment of a human case of sleeping sickness with tryparsamide can make the strain resistant to arsenic." He finds resistant strains comparatively common in Nigeria in untreated natives, even in districts where there is no record of any sleeping sickness treatment having been carried out.

It was during an investigation of thc degree of resistance to arsenic possessed by various human strains in man in the West Nile area of Uganda that the first and only instance of *T. rhodesiense* was detected in that Protectorate. Three strains were obtained from patients who had resisted a full course of tryparsamide. Two of these strains proved to be *T. gambiense* and one *T. rhodesiense*.

VAN HOOF writes also of Bayer 205 resistance, which he finds very difficult to induce in *T. gambiense*. He has not succeeded in inducing a resistance greater than 0·10 gramme per kilo. body weight. The drug is cumulative in effect, and great care is needed in the treatment of the animals. The acquired resistance moreover appears to be unstable and may disappear in a single direct passage of the strain to another host, an experience also common in attempts to make *T. gambiense* resistant to arsenic. Apparently acquired Bayer resistance is reduced also by cyclical passage of the strain through tsetse. The Belgian investigators conclude that the relapse strains after drug treatment are not only composed of drug-resistant survivors, but of normal trypanosomes that have escaped contact with the drug owing to their inaccessibility in the body.

I believe that the behaviour of *T. gambiense* when exposed to gradually increasing doses of arsenical drugs is connected in some way with its mild character in the mammal. In the monkey and man, for example, a chronic and ultimately fatal disease is commonly set up by this trypanosome. In the course of its life in such a host the trypanosome has to contend with wave after wave of " antibody," and in the struggle its constitution is changed. For one thing,

its transmissibility by tsetse is diminished. The imposition of fractional treatment with a trypanocidal drug accentuates and hastens this change, and so we get the resistant but non-transmissible product.

I have suggested recently (DUKE, 1935b) the abandonment of the study of old laboratory strains and their replacement by strains freshly isolated from their natural hosts in Africa. This suggestion did not imply any kind of disparagement of the valuable work that has been done with the ancient curiosities of many European laboratories. It was intended as a reminder that what we are actually called upon to control in medical and even in veterinary practice in Africa are, in the main, strains that are dependent on intermediate hosts for their propagation. After becoming adapted to life in small rodents in the laboratory, these strains often change considerably in various biological respects.

IV.—THE POWER OF TRYPANOSOMES OF THE *brucei* GROUP TO INFECT MAN.

(*a*) EXPERIMENTS IN VITRO.

Numbers of attempts have been made during the last 30 years or so to explain man's remarkable resistance to the pathogenic mammalian trypanosomes, and why and how he succumbs to their invasion. From time to time, there have been instances of accidental infection of laboratory workers, sometimes with strains reputed to be non-pathogenic to man. There have also been a number of unsuccessful experimental inoculations of living *T. brucei* from animal sources into man. But it was not until YORKE, ADAMS and MURGATROYD (1929), in Liverpool, discovered how to keep the trypanosome alive outside the body that any real progress was made. It is not necessary to describe their method here. It will suffice to quote briefly certain of their findings.

Laboratory strains of *T. equiperdum* and *T. rhodesiense* were killed by human serum diluted to 1/5,000 and 1/25,000 respectively, and *T. congolense* succumbed to a 1/10 dilution. An old laboratory strain of *T. gambiense*, on the other hand, resisted pure human serum.

Certain normal sheep and rabbit sera exerted an anti-trypanocidal action. It was found also that in certain pathological conditions such as acute yellow atrophy, the serum loses its normal trypanocidal power. It was concluded that " resistance to the cytolytic action of human serum is a fixed character of *T. gambiense* which survives many years' passage through laboratory animals, but that the serum resistance of *T. rhodesiense* is a ' labile ' character which is relatively quickly acquired and quickly lost." The authors proceeded to put forward the view that the source of both *T. gambiense* and *T. rhodesiense* is *T. brucei*, the evolution of the varieties that infect man depending on the acquisition by certain strains of the power to resist the trypanocidal action of normal human serum. The same main conclusion about the relationship of the three trypanosomes to one another had been expressed by me some years earlier (DUKE, 1921).

Later, ADAMS (1933) published experiments carried out by him in my laboratory in Uganda on freshly isolated strains from various sources, using the Liverpool technique. An abridged summary of his conclusions, as they affect the present discussion, must suffice here.

ADAMS found that there was no demonstrable difference in trypanocidal power between the sera of natives and Europeans. No evidence of susceptibility to the action of normal human serum could be detected in any of four strains of *T. gambiense* studied over a period of 12 months. The serum of a sheep infected with several strains of *T. gambiense* showed definite and rapid trypanocidal action ; serum of a naturally infected native had very little action. The sera of four other more recently infected sheep were also trypanolytic and showed some degree of specificity of action on their appropriate strains. The direct transference of *T. rhodesiense* through small laboratory animals was rapidly attended by a reduction in resistance to human serum ; in four strains susceptibility had appeared by the third, fifth, sixth and eighth passages. A single cyclical passage was followed by a considerable reduction of resistance to human serum ; a second cyclical passage was attended by a further reduction. The resistance of two strains of *T. brucei* to human serum was not so great as would be expected from observations made with an old laboratory strain.

Of special interest were the results obtained with baboon and fowl sera. The baboon is generally admitted to be completely resistant to infection through any ordinary channel with freshly isolated strains of the *brucei* group. Using the serum of a baboon that had completely resisted heavy inoculation with *T. gambiense*, ADAMS found little or no trypanocidal effect *in vitro* against *T. gambiense*, even in the pure serum. The serum of the fowl proved completely devoid of trypanocidal power, although the Uganda fowl possesses a very considerable degree of natural resistance to both the metacyclic and ordinary forms of *T. gambiense* and *T. rhodesiense*.

In the course of his investigations, ADAMS, in September, 1931, tested *in vitro* a strain of *T. rhodesiense* against human serum and found that a dilution of 1/10 destroyed all trypanosomes in between 6 and 12 hours, and a dilution of 1/2 in between 3 and 6 hours. Three months later, CORSON found this same strain actually pathogenic to man, it having meantime been passed cyclically through tsetse. Moreover, CORSON reports that this same strain earlier in its career at his laboratory and 3 months after removal from man, was more sensitive to human serum in subinoculated rats than other strains of *T. rhodesiense* fresh from man and similarly tested.

Assuming therefore that CORSON's serum possessed normal resistance to trypanosomes, ADAMS concluded that the serum factor is not necessarily a criterion of capacity to infect man. He wisely adds that the crucial test must be in man himself.

ADAMS remarks " these observations are of great interest in view of their obvious inconsistency with any thesis that trypanocidal body in the serum

is alone responsible for immunity against trypanosome infection." He inclined to the conclusion that having attained a footing in man, due to initial lowered resistance of the individual, *T. brucei* would perhaps succeed in infecting normal healthy man by direct passage. His experiments led him to believe that cyclical passage tended to reduce the power of survival in man's blood.

Other recent observations on the trypanocidal action of human serum have been recorded. ZIMMERMANN (1931) examined the sera of people suffering from various diseases and found that, undiluted, all such sera retained a trypanolytic action against the trypanosomes of the polymorphic group, although this action was diminished in certain diseases.

FAIRBAIRN (1933) could detect no difference in the trypanocidal action *in vitro* of twenty-nine European and native sera in Tanganyika.

CORSON (1931) found that human serum had greater effect in rats on strains that had been maintained in sheep and goats for some months than on strains direct from man. He also decided (CORSON, 1933) that the resistance of guinea-pigs and of some white rats to *T. brucei* does not seem to be connected with any trypanocidal action of their serum. It is evident that, up to a point, the degree of susceptibility of a trypanosome to human serum shown by the *in vitro* test corresponds to the actual behaviour *in vivo*. There is much to be learnt from these *in vitro* experiments and much that agrees with and helps the interpretation of the results of more recent work on man himself. Some of the discrepancies are, no doubt, explicable by the complexity of the conditions and the number of variable and uncontrollable factors present in the actual tests on man.

It seems to me, however, improbable that the forces that operate in the extravasated serum, and especially in dilutions thereof, are identical with those that confront a trypanosome when it enters the living tissues. I think, too, that it is impossible at present to foretell with certainty from the action of a serum on a trypanosome *in vitro* whether the donor of the serum is or is not susceptible to infection with that strain. If this were possible, the test would indeed be invaluable. I feel strongly, however, that the method does merit further research, the observations *in vitro* being carefully controlled by experiments on man himself.

(b) EXPERIMENTS ON MAN HIMSELF.

(1) Experiments with *T. brucei*.

It is unnecessary here to attempt to enumerate all the recorded instances of exposure, accidental or deliberate, of man to *T. brucei* from ruminant sources. By far the most extensive was the great experiment by TAUTE and HUBER (1919) in which 129 natives were inoculated with *T. brucei* without any infection resulting. In recent years negative tests on several

different strains have been carried out by CORSON; and I have tested eight different strains on man without obtaining an infection (DUKE, 1936).

(2) Experiments with *T. gambiense* and *T. rhodesiense*.

CORSON's own three infections with *T. rhodesiense* include an accidental natural infection, an inoculation with a strain that had passed through fly after its isolation from man and had been maintained for 19 months away from man mainly in goats and sheep, and a fly infection with a *T. rhodesiense* that had been passaged by fly through six sheep during a period of some 17 months (CORSON, 1932, 1936). These experiments demonstrate the infectivity to one individual of different strains of *T. rhodesiense* under different conditions of maintenance. Apart from the work in Uganda, yet another experimental infection of man with *T. rhodesiense* has been recorded; *viz.*, a native in CORSON's laboratory who was infected by fly with a strain that had been for some 10 months in dik-dik antelope.

During my work on man's trypanosomes from 1933 onwards, I have employed altogether 73 volunteers, all save one African natives. A great deal of information has been obtained from these investigations which it is unnecessary to discuss fully here. Some of the facts relevant to this discussion will be given here and others in the section on the game reservoir (DUKE, 1935, 1935a).

(*a*) Prolonged maintenance of *T. rhodesiense* in guineapigs has on several occasions, and with different strains, been attended by definite impairment or complete loss of the power of the strain to infect man.

(*b*) A strain of *T. gambiense* maintained for 14 years in laboratory animals in Hamburg, proved at the end of that time readily infective to man.

(*c*) A strain from Nigeria, in character half-way between *T. gambiense* and *T. rhodesiense*, was pathogenic to man 3 years after its first isolation.

(*d*) Two different strains of *T. rhodesiense* have been shown to behave differently in the same man. One failed to, and the other did, infect him.

(*e*) Clean flies that had taken their first two meals on monkeys infected with a strain of *T. rhodesiense* that had lost its pathogenicity for man were nourished entirely on human blood during the first 3 weeks of the cycle of development of the trypanosome in their interior. These flies, on the completion of the cycle, were still unable to infect man. (DUKE, 1935a.)

(*f*) It has been found that the appearance of a small tender swelling at the site of the bite of an infective fly is a not uncommon symptom of an infection of man with *T. rhodesiense*. Where subcutaneous inoculations of infected blood were employed the slight local disturbance disappeared rapidly when no infection resulted, but when infection ensued, the local symptoms steadily increased during the last few days of the incubation period and before trypanosomes were recognisable in the peripheral blood.

V.—THE BEHAVIOUR OF MAN'S TRYPANOSOMES IN ANIMALS.

(a) DOMESTIC ANIMALS.

T. gambiense.

In domestic animals this trypanosome is generally mild in its effects and liable rapidly to die out. Certain of the strains examined by the League of Nations Commission were found to be readily communicable to sheep and goats, particularly the former (DUKE, 1928). My own experiments led me to the following conclusions about infection in these two animals (DUKE, 1928a). Natural immunity in sheep and goats varies from complete immunity to a degree of susceptibility in which the trypanosome is a contributory or even possibly on rare occasions the direct cause of death. Transmissibility of *T. gambiense* in the sheep diminishes as the infection progresses. Immunity can also be acquired and the infecting trypanosome may die out. In individual sheep the trypanosome may persist for at least 15 months in a form transmissible by tsetse. Different strains undoubtedly differ considerably in their adaptability to sheep and goats.

In Uganda native sheep appeared to be more susceptible than goats to infection with *T. gambiense.*

Little work has been done with this trypanosome and cattle. What evidence there is points to calves being very unsuitable hosts.

T. rhodesiense.

Most of those who have studied this trypanosome in sheep and goats have recorded its high virulence in these animals. Recently in Uganda METTAM has found that cattle are not so susceptible as was at one time supposed. Several of his animals, after a period of some months when the infection was fairly acute, eventually recovered condition and, in one case at least, threw off the infection. This particular animal was, however, susceptible to reinfection by the same strain a short time after its apparent recovery.

After being for 294 days in oxen this strain still retained its pathogenicity for man. We must, therefore, conclude that whereas sheep and goats die rapidly from *T. rhodesiense*, in cattle this trypanosome may survive for a considerable time in a form still pathogenic to man. Actually it is unlikely that cattle do play an important part as a reservoir of this trypanosome because in game-tsetse areas they cannot survive, but die out rapidly from the effect of other species of trypanosomes also carried by the tsetse, or of multiple infections.

The position seems then to be that sheep and goats may act as a reservoir for certain strains of *T. gambiense*, but their actual importance in this respect is much reduced for two reasons. First, as a rule the trypanosome loses its transmissibility rapidly in these animals. Secondly, as affording food for *G. palpalis* they will help to divert the flies from biting man. Their value as a

source of food for man must also be considered. As concerns *T. rhodesiense*, the virulent form kills these animals rapidly, although, as CORSON has shown, a strain may retain its power of infecting man after a number of cyclical passages through sheep over a period of 17 months.   Cattle are so vulnerable to *T. congolense* and other tsetse-carried game trypanosomes that they are unlikely to play an important part in maintaining *T. rhodesiense* in nature.   With the mild strain it is possible that sheep and goats, which are sometimes found in settlements in game-tsetse country long after the cattle have died out, might serve as a reservoir of *T. rhodesiense*.   It must, however, be remembered that the mildness of these mild strains may be restricted mainly if not entirely to man.

(b) WILD GAME.

Work on this subject was first started in 1909 by the Royal Society's Commission in Uganda under Sir DAVID BRUCE.   Human strains of *T. gambiense* were inoculated into reedbuck and bushbuck in captivity, and the subsequent history of the animals was studied.   These experiments showed that *T. gambiense* could survive in antelope for at least 21 months, but no tests were carried out upon man himself.   During the last year or so I have carried out some more experiments with *T. gambiense* and antelope at Entebbe, and have been impressed by the difficulty of infecting these animals with the typical Eastern form of the trypanosome.   When infection did occur it lasted but a short time.   These results indicate that typical mild *T. gambiense* is unlikely to survive in antelope. The League of Nations Commission, however, found that strains differed considerably in their behaviour in domestic ruminants, and it is probable that the same is true with antelope.   As a rule, the conditions associated with the occurrence in Africa of trypanosomiasis gambiensis will not favour the establishment of this trypanosome in game animals, and we can, I think, safely regard man as the main reservoir in nature of this species.

Turning now to *T. rhodesiense*, a considerable amount of work has been done upon the behaviour of this trypanosome in game.   Some of CORSON's experiments have been mentioned, and his later work will be awaited with interest.   My own results will now be presented in the form of a brief list of the findings that bear on the present discussion.

(*a*) A strain of *T. rhodesiense* fresh from man and readily transmissible by tsetse was introduced into and passed for 18 months through a series of fourteen guineapigs.   At the end of that time the strain had lost its transmissibility by tsetse and it failed to infect a volunteer.   Another line of the same strain, after 98 days in a bushbuck, then 30 days in a fowl and then 294 days in oxen, proved readily transmissible and readily infective to man.

(*b*) A second strain underwent seven consecutive cyclical passages through *G. palpalis*, then two passages by the syringe and finally another cyclical passage,

all save one in monkeys.   When tested in man at the tenth and eleventh passages, it was found to be non-infective.

(c) *T. rhodesiense* may retain its cyclical transmissibility by *Glossina* for at least 28 months in an antelope.

(d) Strains of *T. rhodesiense* differ in their adaptability to antelope.

(e) Individual antelope react differently to the same strain.   There is some evidence to show that bushbuck and reedbuck are better suited to the requirements of the trypanosome than oribi and situtunga.

(f) Strains of *T. rhodesiense* differ in their power of infecting man ; some lose this power more readily than others.

(g) There are some indications that with strains whose pathogenicity to man is neither very stable nor pronounced, the inoculation of the blood forms of the trypanosome is more likely to produce infection in man than the introduction of metacyclic forms by the bite of an infective tsetse.

(h) There is evidence that flies infected from an antelope carrying *T. rhodesiense* may fail to infect man, whereas flies infected from a monkey that has been infected by an inoculation of the same antelope's blood may infect him.

(i) A consideration of all the results obtained during the last few years at Entebbe leads me to conclude that although antelope may remain infected with *T. rhodesiense* for a year or two, there is a definite tendency for the trypanosome in these circumstances to lose its infectivity to man.   It has been noted that whereas tests on man carried out with the blood of laboratory animals infected from an antelope (or with flies infected from them) may all or most of them be positive, when the tests are made with flies infected from the antelope itself the results on man may be very irregular.

(j) *T. rhodesiense* may die out from the blood of an antelope in a few months or may survive for 2 years.   Such an antelope is, however, susceptible to reinfection with another strain of *T. rhodesiense* a very short time after the disappearance of the first infection.

One bushbuck and two reedbuck have undergone reinfection.   The reinfection strain in the bushbuck failed to infect man ; those in the two reedbuck succeeded.

(k) A young spotted hyena was infected with *T. rhodesiense* by fly and 2 years later the animal appeared in perfect health without ever having showed symptoms. 569 days after its first infection its trypanosomes were readily pathogenic to a number of volunteers.   In view of the fact that both dogs and jackals succumb rapidly to *T rhodesiense* this is indeed a remarkable record.

(l) A serval cat, accustomed to captivity when infected, was found to be very susceptible to *T. rhodesiense* and died of the disease in 45 days.

An impression acquired during the conduct of these investigations with man and antelope is the variability both of hosts and of trypanosomes. Obviously it is dangerous to dogmatize, and we must be prepared for a more or less open verdict in the end.

It is now plain from actual experiments that the African big game can support *T. rhodesiense* in a form pathogenic to man for a considerable period. Also that an antelope, having once thrown off an infection, is not immune against reinfection. On the other hand, there are suggestions that successive infections may eventually lead to the establishment of a fair degree of immunity for some length of time. If this is so, the trypanosome will depend largely upon young animals for its spread in nature.

We have seen that strains differ in the stability of their power to infect man, and that in several instances flies cyclically infected from antelope were not infective to man, although the strain when isolated in monkeys could still infect him. It is, of course, by cyclically infected tsetse that the trypanosome reaches man in nature, so that this observation suggests that antelope are not so great a danger as a reservoir of the human trypanosome as has hitherto been supposed.

Fortunately any uncertainty that persists about the extent to which the game acts as a reservoir is of no great gravity. For we know enough already to justify certain practical decisions which will be set forth below.

We now come to the main task before us, namely, the attempt to reach some conclusion about the origin of *T. rhodesiense* and the fate of African big game.

VI.—On the Origin of *T. rhodesiense* and the Rôle of the Game in the Spread of the Human Trypanosomes.

About the origin of *T. rhodesiense* there have for years been two schools of opinion, the German and the British. The German view, put forward by KLEINE and his colleagues, is that *T. brucei* and *T. rhodesiense*, alike though they are, are really different species, and that *T. rhodesiense* is a derivative of *T. gambiense*. The other view, originating with KINGHORN, YORKE and LLOYD, holds that *T. rhodesiense* is merely a variety of *T. brucei* that can infect man.

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trypanosomiasis breaks out in a game-tsetse area it is always of the *rhodesiense* form.

Besides these larger outbreaks there have been sporadic cases recorded from time to time, usually discovered accidentally and to the surprise of all concerned. We are not for the moment concerned with the occurrence in Nigeria of cases of *T. rhodesiense* infection in what is primarily a *T. gambiense* area.

These sporadic cases have been recorded by DAVEY; by BEVAN in Rhodesia; by ARCHIBALD (1922) along the southern Sudan border; and quite recently by LAMBORN and HOWAT (1936) in Nyasaland. ARCHIBALD tells me that in the last month or so, another fatal case has been found in the southern Sudan in a *palpalis*-free area, the symptoms of which point to *T. rhodesiense*.

In 1927, I found an isolated case in the Belgian Congo, just over the Congo-Uganda border; and another in Uganda in 1930.

All the Sudanese, the Uganda and the Congo cases were apparently acute; the Nyasaland and Rhodesian strains were mild in man.

Thanks to Mr. BEVAN's initiative I was able to examine his strain, which was isolated from an old native at Gowe, a small settlement in one of JACK's fly foci; a father and son were found to be infected, and like LAMBORN's case appeared to be " carriers."

This strain proved to be pathogenic to man when tested in Uganda a year or more after its isolation from the native. It showed the characters of *T. rhodesiense*, arsenic resistance and virulence in small animals. A detailed account of the strain will shortly appear in *Parasitology*.

The significance of these mild strains will be discussed later in this section.

(*b*) SOME GENERAL CONSIDERATIONS ON THE *brucei* TRYPANOSOMES IN THEIR
    NATURAL ENVIRONMENT AND ON THE DERIVATION OF *T. rhodesiense* FROM
    T. brucei.

These trypanosomes, depending as they unquestionably do on tsetse for their survival and spread, are in consequence restricted for their mammalian hosts to animals upon which the tsetse feed.

Where man is the sole or principal animal in the flies' dietary in which the trypanosome can survive we find the typical mild form of *T. gambiense*. This form must be taken as representing the highest degree of adaptation yet attained by the *brucei* group for man.

The adaptation is by no means perfect, judged by the standards attained in other groups of trypanosomes. Nevertheless, in its mildest form in man, *T. gambiense* may produce an infection lasting for many years without producing serious disability and from which recovery may take place unaided. Generally, however, the presence of this trypanosome in human communities is associated with a considerable mortality.

Moreover, as we have seen, in man loss of transmissibility is likely to occur, and this change is much commoner in *T. gambiense* than in other members of the group in any of their mammalian hosts.

It would appear, too, that the population of sleeping sickness areas is prevented from increasing because of the tendency, in such circumstances, for the disease to take on epidemic form. There is as far as is known no such tendency to increased virulence in *T. brucei* when its primary host, the game, multiplies in a game-tsetse area.

All these considerations seem to me to indicate that man is, biologically speaking, a secondary host to the *brucei* group; one which, in *palpalis* areas in primitive African conditions, was gradually assuming primary importance.

Turning now to game-tsetse areas, wherein man is usually of minor importance to the fly. First, in uninhabited game country. Here the situation appears to be as follows. *T. brucei* is admirably adapted to life in the big game upon which the fly depends for blood. The majority of strains of *T. brucei* are probably incapable of infecting normal man. The minority that can infect him will, in the conditions normally prevailing in game country, rarely have the opportunity. The power, latent in the group, of using man as a host is thus in abeyance, because man in primitive Africa must have been but a fortuitous host to both fly and trypanosome.

We have seen that different people have different powers of resistance to trypanosomes. Also that this variation in man's resistance is not necessarily dependent on disease, although debilitating factors do perhaps exert an influence.

I believe that in addition to this variation in the susceptibility of man, another important factor exists, namely, differences between different strains of *T. brucei* in their inherent power to infect man.

When one of these " pathogenic " strains meets a hypersensitive individual, infection will result. This event is obviously most likely to occur where human settlement exists in game-tsetse country.

The understanding of these problems has unfortunately been made more difficult by a common attitude of mind towards the reservoir question; for there is a tendency to assume that if a strain of trypanosomes recovered from game is found to be infective to man, that strain must necessarily at some earlier stage in its history have been established in man.

What symptoms will *T. brucei* produce when it first becomes established in man's blood? Hitherto it has been assumed that it will appear as the virulent form of *T. rhodesiense*.

No doubt this is often true. But surely the mild cases of the type described by BEVAN and by LAMBORN suggest that on its first establishment in man *T. brucei* may sometimes prove to be almost as innocuous to its new host as it is to game. And here it may be noted that in the recent work with volunteers in Uganda, instances were observed where *T. rhodesiense* itself assumed a very mild form, at all events at the onset of its infection of man. It was, of course, not possible

to allow these experimental infections to pursue their course untreated. The possibility of *T. brucei* being mild in man from its first onset is apt to be over-looked because the mild form will be much more difficult to detect than the better known virulent form of *T. rhodesiense*. It seems to me, however, easier to explain these sporadic infections in this way, than to suppose that they represent either a " stray " *T. gambiense* or a vestige of a long association between the more virulent type of *T. rhodesiense* and man. Any acceptable theory of origin must be able to reconcile and explain the occurrence of the virulent and the mild types of *T. rhodesiense*. Is it necessary to conclude that one form is derived from the other ?

KLEINE (1928) studied strains of *T. rhodesiense* isolated at Ikoma some years, after the first recognition of the so-called Mwanza epidemic of this part of Tanganyika. He concluded that the trypanosome was gradually becoming adapted to man and more and more like *T. gambiense*.

We see then how the acute may develop into the chronic form of *T. rhodesiense*. The change may take some years, or man may be exterminated before it has time to develop ; for human settlement in primitive African conditions is, at the best, precarious in game-tsetse regions. But this transition from the virulent to the mild form does not account satisfactorily for all these sporadic mild cases. The theory of direct origin from *T. brucei* allows also for the occurrence in sporadic form of the virulent strain. Isolated cases of this type would easily pass unrecognized unless they appeared where settlement was fairly close, for the victim would speedily die and unless several cases occurred it is unlikely that any notice would be taken of an occasional death.

We have learnt, both from *in vitro* experiments and from actual tests on man, that *T. rhodesiense* is liable to lose its power of infecting man. A strain newly established in man might in this way fail to pass cyclically to a second human host. And even if it did survive passage by fly from man to man, the same strain introduced into ruminants might lose its pathogenicity. This has actually happened experimentally with *T. rhodesiense* in antelope in Uganda.

Finally, even when a strain does retain its infectivity to man the chances must often be enormously against the trypanosome actually reaching another human host, under the conditions normally obtaining in typical game-tsetse country. Individual flies do not live long and the infective few will be feeding during their life-time mainly on game. In mild cases, too, the transmissibility of the strain in man may be impaired, as we have seen happen not uncommonly in *T. gambiense*. For these and other reasons the chances of the spread of infection in man in sparsely frequented areas must be very slight.

In discussions on this problem it is often assumed that frequent fly-man-fly passages of a strain will result in an enhancement of its virulence for man. This, however, is contrary to the actual evidence, epidemiological and experimental. Why, indeed, should this happen when, as we have seen, the virulent type tends in these circumstances to become more mild ?

Direct transmission of the trypanosome, which can easily occur in human communities, is a different matter, and I still believe that this factor may have played a big part in the Mwanza epidemic. TAYLOR in Nigeria has also called attention to the possible importance of direct transmission.

So much then for the ways in which *T. rhodesiense* may arise from *T. brucei*.

(c) ON THE DERIVATION OF *T. rhodesiense* FROM *T. gambiense*.

We must now consider the other main alternative, namely, that *T. gambiense* can in nature retrace the steps of its evolution from *T. brucei* and appear as *T. rhodesiense*. KLEINE believes that this may happen when *T. gambiense* is introduced into a human community that has had no previous contact with trypanosomes. An apparent objection to this suggestion is that increase in virulence does not occur when *T. gambiense* is maintained in, for example, *Cercopithecus* monkeys.

Recently LESTER has inclined to the German theory of origin as best explaining the distribution of human trypanosomes in Nigeria ; but he also believes that *T. rhodesiense* may at times arise from *T. brucei* direct. How does the evidence bear upon this view ? We have seen that the characters of a trypanosome are not influenced by the species of tsetse that transmits it. This disposes of a convenient explanation according to which *T. gambiense*, when transmitted by *G. morsitans*, is changed into *T. rhodesiense*.

It seems to me, however, that the frequent occurrence of *T. rhodesiense*-like strains in man in the Nigerian sleeping sickness areas, where at one time only *T. gambiense* was supposed to occur, can also be explained by direct derivation from *T. brucei*. For it appears that in Nigeria man is in close contact with two and possibly three different species of tsetse, two of which at least feed readily on game and must therefore be carrying *T. brucei*. The final verdict on this interesting situation must, of course, rest with LESTER, the man on the spot.

Some interesting observations recently made by VAN HOOF indicate that the species of its mammalian host may influence the character of *T. gambiense*. VAN HOOF has passed *T. gambiense* cyclically by *G. palpalis* through a series of domestic pigs and finds that the trypanosome tends to assume the characters of *T. rhodesiense*. In the earlier passages in the pig, trypanosomes were very scarce, although tsetse were readily infected from them. Later on, parasites became more numerous, and their virulence and transmissibility increased. In at least two of the strains resistance to arsenic was also increased after the pig passages.

My own limited experiments with the wild pig and man's trypanosomes in Uganda led me to conclude that this animal, though susceptible, threw off its infection in a short time. (DUKE, 1933b.)

VAN HOOF's findings are the more important because the pig is one of the few large mammals on which *G. palpalis* feeds extensively in nature. Moreover, in parts of Africa pigs are kept in numbers by natives.

If circumstances should cause man's disappearance from a *palpalis* area, the wild animals to which the fly would turn would be reptiles, hippos, elephant and wild pig ; and it is improbable that the first three can serve as satisfactory hosts for *T. gambiense*.

It will, therefore, be interesting to learn whether, simultaneously with the changes described by VAN HOOF, the trypanosome retains its power to infect man ; also whether it behaves similarly in the wild and in the domestic pig.

There are large areas in different parts of Africa that have been deserted by their inhabitants because of the depredations of the wild pig, which is an extremely common pest both in Uganda and in the Belgian Congo. The damage done by this animal often rivals and completes the destruction to settlement wrought by sleeping sickness, and the deserted overgrown shambas for years afterwards swarm with these animals.

Up to this point we have discussed only the change from *T. gambiense* to *T. rhodesiense* occurring in the absence of man. Another possibility to be borne in mind and about which there is little or no evidence is that *T. rhodesiense* may be just a virulent variety of *T. gambiense* that is found wherever infection of man is extensive.

In this connection it is interesting to note how acute were some of the cases of so-called *T. gambiense* infections of man described by the first Royal Society's Commission in Uganda. Would these strains, if subjected to modern tests, have been classed as *T. rhodesiense* ?

(*d*) FINAL REFLECTIONS.

It is evident from the foregoing that no irrefutable conclusion in favour of one or other method of origin of *T. rhodesiense* can be reached at present. As far as *T. gambiense* is concerned, although in its typical form this trypanosome appears to be unsuited to life in game animals, we must, nevertheless, assume that there are strains that can survive in game for a time at least, and retain their pathogenicity to man. It is, however, probable that game plays but a negligible part in the perpetuation and spread of this trypanosome. Nevertheless, no one nowadays would wittingly allow any person infected with trypanosomes to visit any tsetse area.

The question that does demand an answer is whether " wild " *T. brucei* can invade man ? If it cannot, then there is no serious reason why "clean" natives should not live in contact with game-tsetse, provided, of course, that infected visitors can be excluded. But if *T. brucei* can invade man on occasion, then a definite policy must be adopted about settlement and game-tsetse areas.

It has been attempted in this paper to show that it is both reasonable and wise to assume that where man is exposed in nature to game-tsetse carrying

T. brucei there is a liability of the trypanosome to establish itself in man, in either a mild or a virulent form, as *T. rhodesiense.*

The logical outcome of this belief seems to me to be not exactly, as DAVEY has recently suggested, the conclusion of the Royal Society's Commission in Nyasaland in 1915, that game should be immediately blotted out from the fly country of Africa, but rather that there should be complete segregation of man from game-tsetse. As far as we know, man can take no harm from residing in game country in the absence of tsetse, except in so far as his crops and possibly his stock suffer. True, most game country in Africa is tsetse-infested, but there are areas where game abounds without game-tsetse ; for example, parts of the new Albert Game Sanctuary.

What is, however, essential is the protection of human settlement from tsetse, and in so far as game not only can attract tsetse, but also destroys crops, game must be eliminated from the neighbourhood of human settlement. These considerations appear to outweigh the value of game as a source of food for the native settlers. Much has been done in this direction in the northern Belgian Congo and in the southern Sudan by judicious modification of primitive tribal customs and the concentration of scattered habitations into villages which can then become self-defensive ; similarly, in Tanganyika with the measures devised by MACLEAN. Such measures are, of course, at first a compromise, but they are perfectly adequate to control the disease to within easily manageable limits.

What, then, is to be the fate of the game ? Surely the indications are clear. Where human settlement is necessary in the proximity of game-tsetse, the game in the vicinity must be sacrificed. At the same time the population must be guarded from contact with tsetse. This may sound a mere platitude, but practical experience in the countries mentioned has demonstrated the feasibility of measures on these lines. As long as there is any chance of the inhabitants of these outpost settlements coming into contact with tsetse, there must be thorough medical supervision, conducted on modern, not on the drastic terrifying old-fashioned, lines. Experience has taught that the native soon learns to co-operate in looking after his own welfare.

There still is, and will be for many a long year, plenty of room in Africa for both natives and game, and a cynic might add even for game-tsetse. Meantime, pending this ideal solution it will be necessary to examine much more closely than heretofore natives who in the course of their daily life are likely to come into contact with game-tsetse. This will call for tact and organization to avoid all unnecessary disturbance.

Measures along these lines with the aid of the drugs now at our disposal and the still more potent substitutes we may hope for from future research, will ensure adequate control over any danger that may arise to man from the trypanosomes of the big game of Africa.

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DISCUSSION.

Professor Warrington Yorke : Dr. DUKE has covered an enormous field, in his address this evening.   Of course, that is not altogether surprising, because as he says in the beginning of his paper, he is presenting to the Society the results of the labours of a lifetime—nearly 25 years of almost continuous work in the study of one disease.   May I say at once that I think it is fit and proper that the Council has taken this early opportunity of inviting Dr. DUKE to present to the Society a summary of his work, and that we are very much indebted to Dr. DUKE for having acceded to the request of the Council.

Dr. WENYON has asked me to open the discussion, and I must confess that as I listened to the address I became more and more oppressed by the magnitude of the task which had been set me, because Dr. DUKE has dealt with so many aspects of the subject—many of them very complex—that the ordinary mind becomes fatigued, and possibly a trifle confused, in its endeavour to orientate itself through the mass.   Obviously, therefore, there is only one clear course open to me—and, I trust, to those who are going to succeed me—and that is that each of us should confine himself to the two or three aspects of the matter with which he may have some trifling knowledge, and leave to others the far more numerous problems about which he knows nothing at all.

Acting on that most excellent precept, I propose to discuss two or three points which were raised by Dr. DUKE in the first part of his paper.   Undoubtedly knowledge of the factors which influence the adaptation of man's trypanosome to tsetse is very important.   Though Dr. DUKE has marshalled a considerable number of isolated observations, unfortunately, we know very little about the problem.   He refers to the fact that KINGHORN, LLOYD and I, a long time ago, showed that though the first part of the developmental cycle of the trypanosome —that is, the part which occurs in the gut of the fly—can take place at low temperatures, the crucial part of the cycle requires much higher temperatures for its accomplishment.   So far as I know, that observation has never been disputed ; and it explains many points concerned with the epidemiology of the disease.   It also explains an interesting historical fact, namely, that the Royal Society's Commission—of which I see at least one member present to-night— which had ensconced itself on the top of a hill in Nyasaland, did not find it so easy to transmit *Trypanosoma rhodesiense* by *G. morsitans* as did those of us who laboured in the heat of the valley.   TAYLOR's experiments were extremely interesting, but, unfortunately, no one seems to have been able to confirm them.   I do not know why that is ; perhaps it is because we have worked with different tsetse flies.   The fact remains however that we have not been able to confirm them.

Dr. DUKE was, I think, the first to draw attention to the fact that trypanosomes may become non-transmissible.   I remember his very laborious experiments—laborious because it is necessary to use such large numbers of flies to

prove the point—and I asked myself by what mechanism does a strain become non-transmissible ? There arose a very definite difference of opinion between TAYLOR and DUKE. TAYLOR seemed to have the idea that if the extra-peritrophic space once became infected, the cycle of development would inevitably complete itself, provided the fly was kept sufficiently long at a suitable temperature. DUKE, however, held the view that certain strains would never complete their development in the tsetse, even though the trypanosomes succeeded in establishing themselves in the extra-peritrophic space.

I think there is one defect about DUKE's recorded observations, namely, that he confined himself to observing gut and salivary gland infections and ignored the state of the proventriculus. TAYLOR evidently attached great importance to invasion of the proventriculus, which he apparently considered to be almost synonymous with salivary gland infection. My colleagues and I asked ourselves the following question : " If the proventriculus becomes infected, does the salivary gland invariably become infected, provided the fly lives long enough ? " At first I thought it did, but recently I have satisfied myself that this is not the case, and that a certain succession of events occurs during the loss of transmissibility by a strain. The first stage is that the trypanosome will not go beyond the proventriculus ; the next is that it does not reach the proventriculus, but remains limited to the gut, outside the peritrophic membrane ; and the final stage is that the parasite is unable to establish itself outside the peritrophic membrane.

The next point to which I wish to refer is this question of drug-resistance. I cannot add very much to the summary which Dr. DUKE has given, but I can add a little. We find that arsenic resistance—and by that I mean resistance to aromatic arsenicals—when once acquired apparently persists indefinitely. I use that phrase or expression in a guarded sense, but it certainly persists for 7 years or more, and it persists through repeated cyclical transmissions by Glossina. Curiously enough, the resistance to Bayer 205 is something quite different ; it is difficult to make a strain resistant to Bayer 205, but you can eventually do so. We made a strain so resistant to Bayer 205 that it withstood 400 times the amount of the drug which is normally required to clear the blood of trypanosomes. As we passed that strain on through mice, it quickly lost that resistance, and now, it appears to have regained almost entirely its pristine sensitivity to the drug.

I have had no experience of making T. gambiense resistant to arsenicals, but I cannot believe that it does not become resistant ; if it does not, it is a strange anomaly. I am not quite sure what Dr. DUKE means, but there is a suggestion in his paper that he believes that the acquirement of arsenic resistance by T. gambiense would imply that it would lose its capacity of being transmitted by Glossina. Of course, if it were true, but I doubt it, arsenic resistance in this infection would have no practical significance whatever. Some years ago we obtained a freshly isolated strain of T. brucei, and we made one branch of it

resistant to tryparsamide—and, therefore, to all the arsenicals and also to the antimonials. We transmitted that resistant strain through guineapigs, partly by blood inoculation and partly by *G. morsitans.* The parent normal strain was maintained in exactly the same way. Both the strains—arsenic-resistant and normal—eventually became non-transmissible at about the same time. We concluded from this work that the arsenic-resistance had nothing to do with the loss of transmissibility.

I was very much interested in the summary which Dr. DUKE gave us in Section IV on the power of *T. brucei* to infect man. It has always been a mystery to me—as, I suppose, it has been to many other people, too—why man should be so resistant to the genus trypanosome as a whole. It was with considerable joy we discovered what we thought to be the explanation of it, namely, that human serum, even when considerably diluted, would kill, in the test-tube, the ordinary pathogenic trypanosomes, except *T. gambiense*; and we reached from these, and other observations, the conclusions which Dr. DUKE has summarized in his paper.

I am not sure about Dr. DUKE's position on this question, or rather I was not sure of his position when I read his paper in the train on my way here. In one part of his paper he seems to doubt whether the trypanolytic substance in normal human serum has anything to do with man's resistance to *T. rhodesiense*; and yet, later on we read, " We have learnt, both from *in vitro* experiments and from actual tests on man, that *T. rhodesiense* is liable to lose its power of infecting man." I thought, when reading the proof of his paper, that those two passages contradicted one another, but on listening to Dr. DUKE's remarks to-night it has become plain that Dr. DUKE, has been unable as yet to crystallize his ideas on the subject. But, like Dr. DUKE, I cannot believe that this remarkable power of human serum is quite unconnected with man's immunity to most trypanosomes.

I have not time to deal as I should like with all the observations bearing on the subject marshalled by Dr. DUKE. FAIRBAIRN infected himself with the contents of a tube in which all the trypanosomes had apparently succumbed to human serum. The explanation, however, must be that the trypanosomes had not all succumbed; it was only a partial trypanocidal effect. We know that *T. rhodesiense* freshly isolated from man is immune to the action of human serum; in FAIRBAIRN's experiment the strain had not been kept sufficiently long in animals to have become completely susceptible to human serum. There is no doubt that individual trypanosomes vary in their sensitiveness to trypanolytic serum, just as they vary in their sensitiveness to drugs in the test-tube. Although we have examined the point in respect of drugs we have not yet ascertained the range of variation of resistance of individual trypanosomes to human serum.

I will now make way for others, and will conclude by saying that my faith regarding the relationship of *T. rhodesiense, T. brucei* and *T. gambiense* is quite simple; I think they are all essentially the same thing, or, in other words, that they are merely different varieties of the same parasite. And I still hold

to-night as true, what I stated with some force in this Society 20 years ago, that the combination of man and fly and game is not a desirable one.

Miss Muriel Robertson : First of all, I should like to congratulate Dr. DUKE on the very wide scope of his paper, which represents a very clear view of what we may call the indictment against the trypanosome and is the result of an enormous amount of work. From the point of view of one who has seen this work in the early days in the field, and who has followed it since, it seems to me that we have now, in regard to this trypanosome work, to get a different angle of approach on the problem and that it is necessary to get nearer to what is actually happening. The field observations on animal reservoirs, transmissibility and so on have been brought to a practical and useful stage. And now, in order to make further progress with these problems, we have to bring them under a much more severe and close observation in the laboratory, and some of the methods illustrated in other work must be applied here.

For instance, we say " this strain is transmissible," " this strain is not transmissible," but we are still ignorant of what the qualities of the transmissible trypanosomes are, or in what way they differ from the non-transmissible. I have read Major BROWN's work on the electric charges of trypanosomes and think it is very illuminating. It will no doubt be further developed as a means of at least definitely fixing one difference to the organism itself. He has shown that trypanosomes can be differently charged ; sometimes they are positively charged, sometimes negatively ; he has also noted the extremely interesting phenomenon that serum made with a positively charged organism will only attack positively charged organisms ; and the same with negatively charged ones ; also that cultivation is at present a feature of the negatively charged organisms. The things implied in this work are very fundamental and do suggest at least some actual characters of the organisms that can be studied in relation to the problems raised in this paper.

I would mention, in passing, that the work now being done in chemotherapy is not only important from the point of view of the practical use of these drugs, but is also proving illuminating from the point of view of the biology of the trypanosome. It is gradually being shown that these drugs have a direct action on some particular aspect of the physiology of the trypanosome. It is interesting to find that the very efficient drug Bayer 205, against which it is notoriously difficult to make trypanosomes fast, attacks so fundamental a function as the sugar metabolism of the organism. It is the following up of indications of this kind—that is of actual reactions and properties of the organisms—which will lead us to an understanding of the problems in the field. The work in the field has been of immense value and has shown where the problems lie and what they are. It seems to me that there is hardly a group of statements in this paper which is not, at times, contradicted by other observations, and indeed by the same observer, in regard to infectivity, virulence, transmissibility, etc. This seems

to me to suggest that we have reached the point at which the problems need some other kind of explanation than can be obtained by experiments on large animals in the conditions in which Dr. DUKE has been working.

Dr. C. C. Chesterman : I have some hesitation in speaking, as my experience has been clinical and directed to the prevention of the disease, rather than to these deeper problems of the biology of the fly and trypanosome.

What is the practical outcome of what Dr. DUKE has told us to-night ? He agrees with us as to the importance of *T. gambiense* infections, which at present account for more deaths than does any other form of trypanosomiasis in Africa, and admits that there we need only to consider the human being and his fly neighbour. What is the practical bearing of his work on *T. rhodesiense* ? It seems that some game animals can harbour *T. rhodesiense*, and can probably be a source of infection to human beings but that human "carriers" are of far greater importance. In supposing the possibility of human infection by *T. brucei*, we are still, however, in the realm of hypothesis. In 1925, when watching a cricket match at Tabora, I had the honour of being presented to Sir Donald Cameron, Governor of Tanganyika, and, in discussing the problem I hazarded the suggestion, " We shall never get it settled until we do human experiments on a large scale." He replied that that could not be done. But one of his own medical officers has since then infected himself twice, as well as a native volunteer ; and Dr. DUKE has also persuaded a large number of volunteers to submit to infection. The experiments were fully justified, but they have not yet given the answer to the question : Can *T. brucei* infect man ? The fly and man have been in contact in Africa for centuries, as long probably as the contact between the mosquito and the malarial parasite and man in malarial infections. Yet we still find a remarkable degree of specificity ; and I think there is hardly any malarial parasite of an animal that can infect human beings So it seems to me, on this analogy, that we must not be certain that *T. brucei* can infect human beings. We know that two spirochaetes, those of yaws and syphilis, are so similar that they have been proclaimed as one, but the latest serological evidence is to the effect that they can be differentiated, while their behaviour to drugs is different, especially their response to mercurials.

With regard to drug resistance and its practical bearing on treatment, I still believe that cases of *T. gambiense* infection can be rendered resistant to all arsenical treatment by improper dosage, because in my experience, later cases in epidemics are harder to treat than are the earlier cases in the same epidemic. That suggests to me that a certain amount of resistance has been created in one strain by some cases which have had either insufficient or irregular treatment.

I would like to ask Dr. DUKE a question which is suggested by the statement that young animals are more likely to have virulent trypanosomes than older ones. It is whether children are sometimes responsible for the continuance of

virulent epidemics.   We know that malaria is kept going by children, probably yellow fever also, and helminthic infections are perpetuated by the young. Certainly children suffering from trypanosomiasis are more difficult to treat than adults ; they have the disease more acutely and larger doses are required to cure them.   When Dr. Duke says virulent epidemics arise where population is large, may not that be largely due to the children ?

What I have gathered from this paper to-night is, that it is more important to supervise human beings than it is to worry about flies and game ; it is easier to control humans and to follow them up ; and, with the rural medical service in the Colonies, this seems still to be the main point of attack.

Dr. J. B. Davey : It is many years since I had any direct connection with sleeping sickness work, therefore, I can do no more to-night than offer a few remarks. But I would take the opportunity of mentioning that, when I last had anything to do with it, it was on the occasion of the Mwanza epidemic when Dr. Duke came over and lent us his valuable aid in dealing with it.   That was a great help to us all.

In Dr. Duke's paper, as I have seen it in proof form, he has, I think, been a little hard on me. He criticizes my suggestion that we might revert to the Royal Society's recommendation about dealing with game, and he says that we advise that it should be *immediately* blotted out. I have looked up the recommendation of the Royal Society, and I see it is " early and complete " blotting out *in fly areas* which is recommended by that body.   I am in agreement with the statement that *immediate* blotting out would be a task of considerable magnitude. But I feel strongly that, apart from immediate or slow blotting out, game is so much protected that it is actually on the increase, and in a country like Tanganyika, where two-thirds of the country is infested with tsetse-fly, something must be done about this, because we cannot afford to give up two-thirds of the whole country to tsetse-fly and game. What is important in order to make the Colonies a success is to grow native human beings, then those natives will grow crops which will tend to make the countries prosperous.   And, instead of having to run a railway at a heavy loss, it would be profitably used for carrying the produce of the land.   We want to increase the area occupied by natives, but if we are to resign two-thirds of a country to the tsetse-fly, which is spreading, we cannot do much in increasing the areas under cultivation.

Sir Walter Johnson : I am afraid I can only speak from the point of view of the rather large epidemic of sleeping sickness which is going through Nigeria at present, and the tremendous importance in Nigeria of the alteration in the virulence of trypanosome infection in man which has occurred there.   Towards the end of Dr. Duke's paper reference is made to a matter which makes my flesh creep, and that is, that herd transmission may be taking place and so increasing the virulence of the strain.   In Nigeria we have a chronic form of

trypanosomiasis, which was described by MacFie as far back as 1912. It is a mild form of *T. gambiense* infection which still persists over a large area of the country. But in those areas in which 3 per cent. to 5 per cent. of the people show this mild form, we are still liable, unexpectedly and at any moment, to get a sudden outbreak of acute trypanosomiasis, which is very fatal.

Owing to increased and increasing facilities for travel and the " Pax Britannica," people are moving about the country more than formerly, and new strains are introduced into those areas by man.

Another possibility is that a former mild strain is being transmuted into a virulent one. During 1935 our sleeping sickness teams in Nigeria diagnosed sleeping sickness in over 80,000 natives. Many of them have the mild disease, but over an increasing area the disease is assuming a violent form. This is taking place in the *G. palpalis* and *G. tachinoides* areas, while the *G. morsitans* areas are bordering them in some districts but by no means in all. The infections are mostly of the *T. gambiense* type. This variation of strain, whether due to a freer movement of the population or to some other form of transmission is, therefore, extremely important in Nigeria. On that account I am very much interested in the suggestions which Dr. Duke has made in regard to this question.

Dr. C. A. Hoare : One of the questions discussed in Dr. Duke's interesting communication concerns the affinities of the three polymorphic trypanosomes. In the absence of any morphological difference between these trypanosomes their identity is based solely on biological characters, such as relative virulence, choice of mammalian hosts, clinical behaviour, and so on. It is not the structure but these biological features which are used as criteria for the three trypanosomes in question.

However, according to the accepted rules and traditions of zoological and botanical taxonomy, the essential criterion of a systematic unit, such as species, is the morphology of the organisms, the classification of which is based on the difference of structural characters between them.

The biological properties of the organisms are—from the taxonomic point of view—only incidental attributes, and of no systematic value, though they serve to define the physiological or ecological peculiarities of the organisms. However, these peculiarities provide supplementary characters of intraspecific value. From this point of view—and it is the only one acceptable to systematists —the three polymorphic trypanosomes represent a single species, *Trypanosoma brucei*, divided into three biological races or varieties : *brucei*, *gambiense* and *rhodesiense*. There is no harm in continuing, for practical purposes, to call these trypanosomes by their old names, as long as it is borne in mind that one is referring to races of the same species.

The advantage of this understanding is that it serves as a constant reminder of the close relationship and essential unity of these three forms, and it separates the group as a whole from the other pathogenic trypanosomes.

Another point to which I should like to refer is the question raised by Dr. DUKE as to whether the degree of adaptation of the different trypanosomes to their insect hosts affords an index of the antiquity of their mutual association. I think there can be little doubt that this is actually the case, namely, the higher the degree of transmissibility in the vector, the more perfect the adaptation between the insect and the parasite, and hence the older—in the evolutionary sense—the association between them. Thus, it is almost certain that the trypanosomes of the *lewisi* group are phylogenetically the oldest, since their cycle of development in the insect and the contaminative method of infection are similar to those of the herpetomonad flagellates in insects. The antiquity of these trypanosomes and the perfect adaptation between them and their vectors is reflected in the rate of infection in the latter, which is the highest for all mammalian trypanosomes, reaching up to 100 per cent.

As regards the African pathogenic trypanosomes, it is conceivable that the inoculative method of infection in them originated as the result of an adaptation of the mechanical method of transmission, as in *T. evansi*. And, indeed, all the stages of this adaptation can be traced in the existing species. Thus, *T. vivax* shows the stage when the trypanosome became established in the proboscis of the tsetse only. The next stage of adaptation is seen in *T. congolense*, in which the development of the trypanosome extends to the stomach, but does not always persist there. The final stage of adaptation is reached in trypanosomes of the *brucei* group, which develop in the stomach and utilise the salivary glands for the production of the infective forms.

The infection rate of tsetse flies with these trypanosomes is fully in accord with the views advanced regarding their evolution, for it is highest in *T. vivax*, lower in *T. congolense*, and the lowest in trypanosomes of the *brucei* group.

Major H. C. Brown : There are two points I would like to make clear in connection with the remarks which have just been made by Miss ROBERTSON.

In the first place it was not my work alone, it was the work of my colleagues, Dr. J. C. BROOM and Dr. HOARE and myself. I am sorry Dr. BROOM is not here to-night. Previously some observers had said all trypanosomes were positively charged, others had said that they were all negatively charged. We found that in a mouse, in the case of *T. evansi*, *T. brucei* and *T. gambiense*, they could be either positively charged or negatively charged. It was even possible to have positively and negatively charged trypanosomes in the same mouse. We were able to separate the positively from the negatively charged trypanosomes, and we were able to breed these pure. If you inoculate the mouse with negatively charged trypanosomes they will breed absolutely pure, and will continue to be negatively charged in the mouse. If a relapse occurs in the mouse, that is to say, if the trypanosomes re-appear in the circulation after disappearing for a time they will invariably re-appear with the sign of the opposite charge ; and mice inoculated from these positive ones will breed true through thirty or

forty generations as positive. The host apparently, as far as we can tell at present, is very important in determining the sign of the charge. That is to say, if you take a negatively charged trypanosome in a mouse and put it into a more resistant animal, such as the rat or the guineapig, then directly it appears in that more resistant animal it will have reversed the sign of its former charge. If a relapse occurs in that more resistant animal, the rat or the guineapig, the trypanosome will again reverse the sign of its charge. One can take it from these animals and put it into mice, and it will breed true through forty or more generations as either positively or negatively charged trypanosomes. Cultural forms of *T. lewisi* and *T. brucei* have been negative. *T. lewisi* in the rat has been negative. *T. evansi*, on first investigation, was positive, but later we separated a negatively-charged variant. *T. brucei*, normal strain, was positive to start with, and then we obtained the negative from that. The tryparsamide-resistant strain of *T. brucei* given to us by Professor YORKE was both positive and negative. *T. gambiense* was first positive, and then became negative after relapse.

As regards the question of the susceptibility to arsenicals of the positive and negative variants, *i.e.*, mice infected with positive and negative variants, we took fifty mice and inoculated them with the positive strain of *T. evansi*, and we took another fifty and inoculated them with the same numerical amount of the negative strain. Then 24 hours after injection we gave each animal 20 mg. of tryparsamide. There was a decided difference in the number which became infected. At the end of the first week, of the positives, 12 had shown infection, as against 33 in the negatives. At the end of the second week there were 20 and 45 respectively. The likelihood of that being due to chance is about one in a million. We did another experiment. We had twenty mice with positive, and twenty with negative trypanosomes both having received the same dose of trypanosomes numerically. At the end of the first, second, third and fourth weeks with the negative mice, 0, 7, 9, 12 showed infection ; whereas with the positive mice not a single mouse showed infection. That is to say, that the positive variants in those experiments with *T. evansi* were infinitely more susceptible to the action of tryparsamide, as far as we can tell from these two series of experiments. Several times we have tried to repeat this with *T. gambiense*, but so far have been unable to do so. Whether that is correlated with the difficulty which Dr. DUKE referred to of making *T. gambiense* resistant to arsenic, I do not know.

There is also one other point, that to which Miss ROBERTSON referred, namely, with regard to the serology. As far as we had gone when we published our last paper, the positive and negative strains in mice were serologically distinct; but I think that much more work must be done before we can separate out the different positive and negative serological types.

Professor P. A. Buxton: There is one very small contribution which a student of insect physiology can make to the discussion. Dr. DUKE calls

attention to the curious observation of TAYLOR, who took batches of *G. tachinoides* and gave them infective meals on four consecutive days, and on those days he kept them at a temperature of 37° C., instead of at the laboratory temperature ; again and again TAYLOR's flies, kept at a high temperature, showed a very high rate of infection. Several workers, using *G. palpalis* have failed to obtain this enhanced rate of infection. It might be added that when LEWIS and I were in north Nigeria we worked out the upper temperature limits of survival of *G. tachinoides*, because we thought the point concerned the physiologist. But we could never get the fly to live 24 hours at 37° C., at any humidity. How can we reconcile these apparent contradictions ? We know that a temperature just below 37° C. is very critical for these flies. I believe that at 35° or 36° C. they might live and yet be adversely affected by the temperature ; perhaps Dr. TAYLOR's observation is an important one, right in all essentials but erroneous in the matter of a degree or so. TAYLOR's discovery may be important to laboratory workers, helping them to infect large numbers of flies at will ; it may also have considerable practical importance, because temperatures of about that order of magnitude are the mean temperatures of that part of Nigeria in the hot period of the year. TAYLOR's experiment was, therefore, very nearly a natural one, and it may be interesting with regard to infection of flies under field conditions.

The President, Sir Arthur Bagshawe : More than 20 years ago we received, at the Tropical Diseases Bureau, a communication from the London Office of the British South Africa Company. The Directors had read reports about *Trypanosoma rhodesiense*, and they were concerned because it seemed to associate Rhodesia with sleeping sickness, which, from their point of view, was unfortunate. Could anything be done about it ? A reply was sent to them to the effect that zoological nomenclature was not concerned with such objections, but it was thought likely that before long the name " rhodesiense " would disappear, and would be replaced by what was, to them, the innocuous " brucei." That was the belief at the time; both BRUCE and YORKE thought *T. rhodesiense* was nothing other than *T. brucei.* If that question were raised again, one would have to give a less confident reply. In fact, one sometimes thinks that knowledge of these problems grows so slowly that our children's children will still be discussing them, and that big game, trypanosome and tsetse-fly will still be bulking large in these debates. But knowledge does grow, and at any moment we may be in a position to make definite progress.

Dr. H. Lyndhurst Duke (in reply) : I would like to explain first of all, in reply to Professor YORKE, that my neglect of the proventriculus in my work on transmissibility was due to necessity and not to oversight. Single-handed, I had to carry out some 30,000 dissections a year, and it would have been impossible to dissect this number had the proventriculus of each infested fly been examined.

With regard to Professor YORKE's uncertainty whether I believe that the acquisition by *T. gambiense* of resistance against arsenic and the attendant loss of transmissibility by tsetse are closely associated : I do not regard these manifestations as cause and effect. VAN HOOF and his colleagues, in the last paper they published, expressed the view that virulence, resistance to arsenic and transmissibility, are definitely characters of a strain of trypanosome wherein strains differ from one another, but that these characters can vary independently of one another. One can, for example, increase resistance without necessarily affecting transmissibility or any other quality.

About drug-resistance in *T. gambiense*, I certainly do not hold that it is impossible to make *gambiense* resistant against arsenic; this has indeed actually been done. The apparent fact that during the acquisition of resistance a strain may lose its transmissibility by tsetse I attribute to the effect of the succession of waves of antibody which are put out by the host in the course of its prolonged struggle with the trypanosome. The additional effect of repeated doses of arsenicals tends to accelerate the deterioration of the strain.

In reply to the remarks which Dr. CHESTERMAN made, I must make it clear to the meeting that the employment of volunteers in Uganda was not inspired by any change in my estimate of the value of human life. Our boldness in this matter was due to the fact that we had so much more confidence in drugs than had workers in the old days. I can assure you—not by way of excuse, as it had to be done—that these natives came forward entirely of their own free will. They visited one another in hospital, and they fully understood what they were up against. Moreover, they knew of the ravages of sleeping sickness in Uganda. It is gratifying to know that we had no set-back in any of the natives we treated, although precautionary lumbar puncture was performed months later on certain of the men to make sure that nothing unforeseen was happening.

As to the suggestion concerning the maintenance of the disease by children, which Dr. CHESTERMAN made, I do not think that would apply, certainly not with the *rhodesiense* disease. The proportion of adults infected is always very high, indicating that it is those who go into the fly-scrub and are exposed to tsetse that suffer most, rather than the stop-at-home children. I do not, therefore, think there is a real parallel between the two diseases, malaria and sleeping sickness, which Dr. CHESTERMAN was considering.

With regard to the remarks of Dr. DAVEY, I am sorry if I misquoted him. I certainly did not mean to suggest that the huge areas in Tanganyika which are now infested with tsetse and useless to man and domestic animals should be maintained as a game reserve for all time. I consider that as settlement increases the game must be pushed away into areas where settlement is not yet necessary. It seems to me that for many years there will be areas which can be left alone for the game without interfering with the population of the country. Where game encroaches on settlements the game must certainly be destroyed.

With regard to the remarks of Major BROWN, it is very interesting to me to

learn about the electrical charge of trypanosomes and I am looking forward
to the time when he and his colleagues will be able to give us the results of his
later experiments and so be able to answer many points which at present are
unsettled.   At the present time, as Miss ROBERTSON remarks, there is still much
uncertainty about the cause and nature of many of the phenomena described.
There may be a connection between the various phases of this endogenous
cycle and the charge carried by the trypanosomes.   It will be interesting to know
whether a positively charged trypanosome is able in any circumstances to survive
in the culture tube or in the fly.

Professor BUXTON's remarks interest me greatly as my own experiments
on the effects of temperature led me to explain TAYLOR's results as due to the
effect of the temperature on the fly rather than on the trypanosome direct.

COMMUNICATIONS.

ARE EXPERIMENTS WITH TRYPANOSOMES IN LABORATORIES IN TROPICAL AFRICA VITIATED BY ACCIDENTAL INFECTIONS?

BY

J. F. CORSON,

Research Laboratory, Tinde, Tanganyika Territory.

Apart from avoidable mistakes, it may be supposed that in tropical Africa animals kept for use in experiments with trypanosomes may fail to be reliable owing to their being accidentally infected with trypanosomes. It may be supposed that native oxen, sheep, goats, dogs, fowls and wild animals may have a chronic infection caused by the bites of wild tsetse flies in areas in which they have lived at some time, and that the chronic infection may escape detection. Antelopes, unless they are born on the laboratory premises or in some other place situated many miles from tsetse flies, cannot be regarded as free from a natural infection, and this applies especially to the more resistant kinds in which infection may be hard to detect. Therefore, experiments are planned which do not

require that the antelopes shall be free from a natural infection with animal trypanosomes. My experience of oxen is very small, but as regards sheep, goats, dogs, fowls and monkeys, I think that this danger is more fanciful than real if care is taken. I suppose that certainty cannot be attained unless the animals are bred on the laboratory premises, for BRUCE and his co-workers (1915) found three strains of *Trypanosoma brucei*, of very low virulence, in native dogs in Nyasaland. Their Strain 26 was infective to cattle, goats, sheep, dogs, monkeys, guineapigs, rabbits, rats and antelopes. Three of the four cattle never showed trypanosomes and the fourth was alive after 335 days. Eight of the fifteen goats used never showed trypanosomes and none died of trypanosomiasis, at least, within the nine months of observation. Of eighteen experiments with eleven monkeys, trypanosomes were never seen in fourteen, and no monkey died, at least, during the six months of observation. Fourteen of the twenty dogs died of the infection, two never showed trypanosomes and four were alive after 224, 120, 148 and 131 days respectively. Only one of ten guineapigs showed infection and it was alive after 246 days. Rats were susceptible and all died. A strain of *T. brucei* obtained from Zululand (CORSON, 1934 *a*), though virulent for dogs and rabbits, infected only about half of the white rats inoculated, causing a chronic infection and failed to infect guineapigs. It is possible, therefore, that infection with *T. brucei* might escape detection for months in sheep and goats and even in dogs and monkeys, but wild flies also convey *T. vivax* and *T. congolense* which may help as indicators of exposure to flies. If, however, the animals are bought from known owners who live near the laboratory and are kept for some time before use, and if no accidental infection is found, in the course of years—by signs of illness, microscopical examination of the blood, inoculation of susceptible animals or in experimental transmission by tsetse flies—it is justifiable to feel confident that the animals are free from latent infection. It would be necessary to inoculate blood many times into various animals such as monkeys, guineapigs, dogs and rats, to be certain that no natural infection existed and this would not be practicable, though it would be done in the course of an experiment in doubtful cases or where the exclusion of such a natural infection was necessary.

Mechanical Infection.

This has been done experimentally with biting flies other than tsetse flies many times from 1901 to 1934, but many failures are also recorded. Experimental transmission with non-biting flies (MUSGRAVE and CLEGG, 1903 ; THOMSON and LAMBORN, 1934) has also been effected. Records of the probable occurrence of mechanical infection in nature by the bites of flies are few. BRUCE and his co-workers (1910) thought that cattle, grazing near the laboratory at Mpumu, were infected with *T. congolense* by Tabanidae, but gave strong reasons for excluding *Stomoxys* as a vector. DUKE (1934) concluded that a young reedbuck had been infected with *T. rhodesiense* by *Stomoxys* in the antelope enclosure at Entebbe. ADAMS (1935) found infection with *T. vivax* in cattle

in Mauritius and thought that *Stomoxys nigra* was the most likely vector. He referred to the opinion of ADERS that infection of cattle in Zanzibar with *T. congolense* was probably due to a species of *Tabanus*. MARSHALL, LESTER and JONES (1935) attributed an outbreak of infection with *T. vivax* in a herd of cattle to biting flies other than tsetse flies.

Conditions at Tinde Laboratory.

In laboratories where Tabanidae and *Stomoxys* are at times plentiful, as at Tinde, this question of mechanical infection needs consideration. The seriousness of such an accident will depend somewhat on the nature of the experiments, but the amount of risk can also be judged. At Tinde, two or three times a year, a species of *Tabanus* appears in considerable numbers, entering the houses and laboratory and becoming rather a nuisance by biting people. *Stomoxys* is also present but not so obviously. The laboratory sheep and goats graze on the premises ; rabbits live in open runs in the day time, guineapigs and monkeys are also kept in the open, while rats and mice, though kept in boxes, are not always screened by mosquito gauze. Light and ventilation are provided for many boxes by wire-netting through which biting flies could easily enter. It is the practice to mark all animals used in experiments with colours, picric acid, carbol-fuchsin, methylene blue and, occasionally, malachite green is used, as no reliance could be placed on labelling or marking boxes. Animals infected with *T. rhodesiense* mix freely with uninfected animals of the same kind, yet during five years at Tinde no unmarked animal, so far as I know, has become infected. It has nevertheless been thought advisable to have as few strains of trypanosomes as possible and at present the infected individuals of the flock of sheep, for example, are only infected with one strain, *T. rhodesiense*. I have recently examined stained thick blood films of most of the stock of animals and have found none infected. For guineapigs forty microscopic fields were thought enough, but for sheep and goats and for monkeys, whose neighbours in the monkey park are infected with *T. gambiense*, 200 fields were examined. The animals examined included 320 guineapigs, all the twenty uninfected sheep, twenty-nine of the thirty-one goats and thirteen unmarked monkeys. The rats and mice used in experiments are so many that they serve as controls of accidental infection. A few years ago, however, about 200 rats were examined as a sample and none were infected. An experiment was made (CORSON, 1934 b) to see whether rats were liable to become infected with *T. rhodesiense* through eating the bodies of rats which had died of the disease, and infection in this way was found to be unlikely. The antelopes were not examined as they are not regarded as uninfected.

CONCLUSION.

It seems that confidence may be placed in the trustworthiness of experiments with human trypanosomes in laboratories in tropical Africa if they are situated

in open country several miles from wild tsetse flies, and if tsetse flies are not repeatedly introduced by trains or motor cars. At the present time, however, with the establishment of aerial transport, much of the work which had to be done formerly in sleeping sickness areas of tropical Africa, can be carried out in places where no fear of accidental infection by biting flies need be entertained.

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PORCINE TRYPANOSOMIASIS IN THE GOLD COAST.

BY

J. L. STEWART, *M.C.*, B.Sc., M.R.C.V.S.

Director of Veterinary Services, Department of Animal Health, Pong-Tamale,
Northern Territories, Gold Coast.

It is but recently that trypanosomiasis has been definitely diagnosed in pigs in the Gold Coast.   Though there are several veterinary officers and a well-equipped laboratory, the pig population of the country is largely in the forest, where no veterinary staff is stationed.   Within the last few years, *Trypanosoma brucei* infection has been found in grade pigs in the Northern Territories ; and, this year, *T. simiae* has been identified for the first time in West Africa in a flock of swine near Kumasi, Ashanti, within the forest belt.   It is probable that the latter condition is common.   The types of infection differ clinically and in view of the confusion on the subject of pig trypanosomiasis, definite observations have been taken.

T. brucei infection.

Several outbreaks have been diagnosed by means of blood slides sent to the Pong-Tamale laboratory from farmers and it was known that this type of trypanosomiasis existed.   However, an excellent opportunity for observation appeared in 1934 when seventeen cases appeared among grade pigs at the Pong-Tamale Farm.   The symptoms were characteristic and confirmed the hearsay descriptions received from farmers and others.   Inappetence and weakness of the hindquarters appear, followed by a chronic progressive anaemia. Temperatures were normal and trypanosomes were present in the blood.   An intense infection of the blood was never found.   This form of trypanosomiasis is often masked by verminous broncho-pneumonia caused by infection by *Metastrongylus apri*.   This nematode is very common and seldom causes trouble *per se*, but becomes pathogenic if the host becomes infected by trypanosomiasis or has its resistance weakened by some other factor.   In the open country, *T. brucei* alone has been found in pigs, though *T. vivax* is the common animal trypanosome.

T. congolense infection.

Recently this trypanosome has been found in blood slides sent from the colony.   In each case, two in number, the pig was suffering from obvious verminous broncho-pneumonia.   Further observation of this infection is necessary to determine the exact pathogenicity.   HOARE (1936) states that *T. congolense* does not impair the health of the infected pigs but in these two cases it is highly probable that trypanosome infection broke down the normal resistance to the lung helminths and caused them to become pathogenic.   Both were native pigs and it is likely that European or grade pigs would exhibit definite clinical symptoms following exposure to *T. congolense* infection.

T. simiae infection.

The first instance of this condition appeared near Kumasi in June this year. A Syrian who owned a herd of about 150 head of mixed grade Yorkshire and native pigs lost 100 in a few weeks from a sudden fatal disease. A passing veterinary officer visited the farm, took slides and found the bloods of infected animals swarming with trypanosomes. On examination at the laboratory, I identified the trypanosome as *T. simiae*, a diagnosis which has since been confirmed by Dr. HOARE. An assistant was sent to the farm in order to get numbers of blood slides and to make detailed observations. He arrived there 10 days after the veterinary officer's visit and found that there were seven pigs only surviving, so that over forty had died in 10 days. While he was there, he saw an apparently healthy native pig begin to shiver and then stagger with manifest weakness of the hindquarters until in 15 minutes the gelt collapsed and died 5 minutes later. Blood was at once taken and examined carefully to eliminate anthrax. Trypanosomes were swarming, and when it was evident that the disease was not anthrax, an autopsy was done which showed good general condition, enlarged spleen, congested liver with small abscesses, congested lungs, small abscesses in the stomach wall and intestines and acute inflammation of these organs. Experimental treatment by means of antimosan was tried but as the assistant had to return to the laboratory it was not possible to observe results and the owner has not since communicated. The Syrian stated that all the deaths had occurred quickly in much the same manner as the one observed by the assistant. The assistant took blood slides of five of the the remaining six pigs, all of which appeared to be quite healthy. On examination, all proved to be infected by *T. simiae* though the slides taken from the dead pig proved to be much more heavily infected. It would appear probable that no clinical symptoms occur in infected pigs until the infection reaches a very high degree of intensity and then the animal would appear to die of an acute septicaemia in much the same way that anthrax affects cattle.

Detailed examination of the slides at the laboratory showed all the forms of *T. simiae* shown in HOARE'S paper (*loc. cit.*) on the subject, with the exception that forms with free flagella were not observed ; Dr. HOARE suggests in a letter to me that it is possible that the forms appearing in column 4 of his Table III actually have no free flagellum but only appear to have one.

The infected pig farm is situated in the Ashanti rain forest and an examination of the area carried out by two of the department's fly catchers showed that *Glossina palpalis* was common round the farm and in the grazing areas.

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NOTE ON *TRYPANOSOMA SIMIAE* FROM AN OUTBREAK AMONGST PIGS IN THE GOLD COAST.

BY

CECIL A. HOARE, D.Sc.,

Protozoologist to the Wellcome Bureau of Scientific Research, London.

In a recent paper (HOARE, 1936) an account was given of all the recorded cases of acute porcine trypanosomiasis in Africa, attributable to *Trypanosoma simiae*, and it was noted that, with one possible exception, they were all distributed in the east-central region of the continent.

The only epizootic outbreak reported from West Africa occurred in Mamou, French Guinea. From the description given by ALDIGÉ (1920) it was concluded that the disease was due to *T. simiae*.

An outbreak in the Gold Coast described by STEWART in the present issue of the TRANSACTIONS (p. 313) represents the first authentic record of *T. simiae* infection in West Africa. It thus extends the distribution of this trypanosome and, furthermore, lends support to my interpretation of the aetiology of ALDIGÉ's case.

TABLE.

T. simiae FROM PIGS IN THE GOLD COAST.

DISTRIBUTION (PER CENT.) OF FORMS WITH RESPECT TO POLYMORPHISM AND FREE FLAGELLUM.

Variety of Forms.			Free Flagellum.			Number of Trypanosomes Examined.
simiae-typical.	*rhodhaini*-like.	*congolense*-like.	Absent.	Apparent.	Present.	
91·0	7·0	2·0	96·0	3·2	0·8	584

Capt. J. L. STEWART was kind enough to supply me with a collection of stained and unstained blood-films taken from infected pigs at Kumasi, Ashanti. Having confirmed Capt. STEWART's diagnosis of *T. simiae*, I availed myself of the opportunity to make a further study of this trypanosome, with the object of checking my previous findings regarding its specific characters (HOARE, 1936). A critical examination of preparations from fresh cases, and especially from new localities, was desirable in view of the fact that in *T. simiae* we are dealing with a species, the position of which among other trypanosomes pathogenic to domestic animals has only recently been recognized.

The appearance of the trypanosomes from the Ashanti pigs is shown in the text figure. They exhibit the same polymorphism as *T. simiae* from other parts of Africa, *viz.*, the classical *simiae*-forms with a well-developed undulating

membrane (Figs. 1 to 4), the slender (*rodhaini*-like) forms with an undeveloped membrane (Fig. 6) and *congolense*-like forms (Fig. 5). As is shown in the accompanying table, the proportion in which these forms occur also agrees very closely with the corresponding figures for the East African strains (cf. HOARE, *loc. cit.*, Table II). The measurements of the West and East African strains of *T. simiae* are likewise within the same range. There remains the question of the free flagellum. This was found to be definitely absent in 96 per cent. of the trypanosomes examined ; in 3·2 per cent. a free portion of the flagellum is apparently (but by no means certainly—see argument in HOARE, *loc. cit.*, p. 632 *et seq.*) present, while only in 0·8 per cent. does a free flagellum seem to be actually present (Fig. 7). If these figures (see table on page 315) are compared with those for the eastern strains (cf. HOARE, *loc. cit.*, Table III), it will be seen

that in the West African strain the absence of a free flagellum is more universal. However, in my opinion, this is not due to any difference between the various strains, but to the fact that the examination of the Gold Coast strain was based on

Trypanosoma simiae FROM THE
GOLD COAST.
1-4. Predominant "*simae*"-forms ;
5. " *congolense* " - like form ; 6.
" *rodhaini* "-like form ; 7. Form
with free flagellum.
(Drawn with the aid of a camera
lucida, at ×2000).
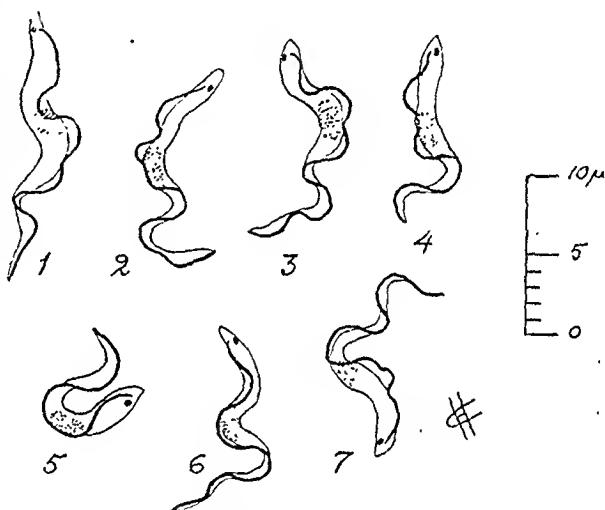
well-stained *recent* preparations, while those examined by me previously were several years old. In the fresh films the delicate portion of the undulating membrane which runs to the tip of the flagellum and which becomes obliterated with time and in defective preparations, can be detected in the majority of the trypanosomes, leaving only a small number (0·8 per cent.) in which there appears to be a short free flagellum.

It is thus seen that both qualitatively and quantitatively *T. simiae* from the Gold Coast pigs reveals the same features as the other strains of this trypanosome described by me. This fact provides further evidence that the polymorphism of *T. simiae* is not due to mixed infections, but represents a constant characteristic of this species.

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SCHISTOSOMA HAEMATOBIUM AND ITS LIFE CYCLE IN IRAQ.

BY

E. A. MILLS,

C. MACHATTIE

AND

C. R. CHADWICK,*
Baghdad, Iraq.

CONTENTS.

I.—INTRODUCTION.

In the *British Medical Journal* of December, 1899, there appeared the following communication from P. S. STURROCK, M.B., B.C., of the Church Missionary Society, Bagdad, Turkish Arabia.

" In *Tropical Diseases*, by Patrick Manson, M.D., F.R.C.P., published by Cassell and Co., in 1898, the geographical distribution of bilharzia haematobia is stated to be limited to Africa and its island dependencies. I regret to say the disease is widely spread throughout Mesopotamia, occurring in those living in towns and villages situated on the banks of the rivers Tigris and Euphrates. I have been able to trace it up to about 900 miles from the mouth of the united rivers, but so far no patients have applied for treatment who dwell upon the river where the influence of the tide of the Persian Gulf is felt. Vesical calculus is more prevalent above Bagdad, where apparently there are more cases of bilharzia haematobia."

The above reference by STURROCK (1899), constitutes the first published information regarding schistosomiasis in Iraq.

No further attention appears to have been paid to the disease until the War. Up to the autumn of 1917, the parasite was found only twice amongst British and Indian troops ; and in both cases, eggs were found in the urine of two sepoys stationed in the Nasiriyah district on the Euphrates. Early in November, 1917, the importance of the subject from the point of view of military sanitation was realized through a small outbreak of bilharziasis among the personnel of an Indian general hospital at Basrah. Capt. C. L. BOULENGER was directed to report upon the origin of this outbreak, and by means of a rapid survey to investigate the prevalence of the disease among the Arab population of the occupied districts. In addition, BOULENGER (1919), made a malacological survey of both the Tigris and Euphrates areas in the hope of discovering the molluscan intermediate host of the parasite.

BOULENGER (1919) reported : " The result of an investigation of the Arab population of Mesopotamia showed that the disease was common throughout the country both in the Tigris and Euphrates districts, the average infection by *Schistosoma haematobium* was approximately 20 per cent. of the male Arabs examined." He made a tour of the country and found the distribution of *S. haematobium* in different districts to be as follows :—

District.	Number of Arabs Examined.	Number of Positive Findings.	Percentage.
Basrah	50	9	18
Kurna	13	11	85
Amarah	30	6	20
Baghdad	24	2	8
Samarrah	20	2	10
Felujah	17	6	35
Baqubah-Sharaban	20	—	—
Totals	174	36	20

The two cases at Baghdad were from the general hospital and it was not definitely ascertained whether infection had been contracted at Baghdad or in the surrounding districts.

With regard to the snail host BOULENGER (1919) writes : " Since bilharziasis was shown to be quite common among the Arab population of Mesopotamia, it became of the greatest importance to examine the molluscan fauna of that country, with the object of ascertaining whether *Bulinus contortus* and *Planorbis boissyi* occur in the occupied districts, or if other gastropoda are acting as the first hosts of the parasite in their absence." BOULENGER searched for *Bulinus* especially, and remarks : " In this respect the results of my investigation were decidedly disappointing, except in a sub-fossil condition no species of the genus were found there." He dissected large numbers of *Neritina, Melanopsis, Lymnaea,* a *Planorbis, Melania tuberculata, Corbicula* and species of *Unio,* but none were found infected with bilharzial cercariae. BOULENGER found dry but fresh shells of *Bulinus contortus* at Amarah, Ramadi, Zeur near Baghdad and Felujah ; he concludes: " My malacological work in Mesopotamia proved somewhat disappointing, much certainly remains to be done on the molluscan fauna of that country. The above section shows, however, that *B. contortus* does occur in Mesopotamia, and, judging from the numerous semi-fossil specimens, must in fairly recent times have had a wide distribution. It is difficult to reconcile the rarity of its present occurrence with the comparatively frequent *S. haematobium* infection amongst the Arabs. Infection experiments should certainly be conducted on some of the other common gastropoda of lower Mesopotamia." BOULENGER finally concludes : " The mollusc *Bulinus contortus* known to be the second host of the parasite in Egypt, was found in Mesopotamia, but does not seem to be of common occurrence in that country."

" The question of the occurrence of *S. mansoni* in Mesopotamia has not been touched on in this report as I have had no opportunity of examining faeces from Arab inhabitants, only two cases of rectal bilharziasis came under my notice during my stay in the country. Both of these proved to be due to *S. haematobium* and the faeces showed the typical terminal spined ova of that species. The abundance of *Planorbis* everywhere suggests that a suitable host exists in Mesopotamia to ensure the spread of *S. mansoni.*"

Referring to BOULENGER's observations, KEMP and GRAVELY (1919) point out that MOUSSON (1874) had already recorded the occurrence of *B. truncatus* Aud. (under the name of *Physa (Isidora) brochii* var. *approximans*) from the banks of the Euphrates, and summarise the war period position with regard to the snail host of *S. haematobium* in Iraq as follows :—

" That Captain BOULENGER's extended investigations failed to result in the discovery of a single living example of *B. contortus* would tend to indicate that the mollusc, owing perhaps to some abnormal climatic conditions, has recently become very scarce in Mesopotamia. If this be so, the parasite must have adapted itself to some new molluscan host, for an outbreak of schistosomiasis of undetermined origin occurred among the personnel of an Indian general hospital at Basrah in November, 1917. Captain BOULENGER states that the only abundant mollusca in water adjacent to the hospital where the outbreak took place were species of *Neritina* and *Melanopsis.*"

HALL (1925) as a result of his investigation, came to the conclusion that schistosomiasis was exceedingly prevalent on the banks of the Euphrates from Hindiyah to the sea, particularly in the rice field areas. In Diwaniyah, he found 80 per cent. of the population affected with bilharziasis, and at Basrah the urine of 711 school children showed 334 infected, *i.e.*, 47 per cent.

HALL remarks that the Mohammedan children are, in proportion, more affected than the young Christians or Jews, and ascribes this to the Mohammedans' more frequent bathing and washing. The following figures are given in support of this statement : Mohammedan children affected, 57 per cent. ; Christians, 30 per cent. ; Jewish, 27 per cent.

NEVEU-LEMAIRE (1929) reviewed the existing literature and analyzed the *Iraq Health Service Reports*, particularly the first quarterly report of 1928 which was as follows :—

District.	January.			February.			March.			Total.
	In Town.	In Dis-trict.	Total.	In Town.	In Dis-trict.	Total.	In Town.	In Dis-trict.	Total.	For 3 Months.
Basrah	2	158	160	—	225	225	6	175	181	566
Diwaniyah	—	25	25	1	49	50	2	58	60	135
Muntafiq	7	1	8	12	7	19	19	5	24	51
Dulaim	—	19	19	—	16	16	—	6	6	41
Baghdad	1	—	1	2	10	12	3	24	27	40
Erbil	—	—	—	—	7	7	—	16	16	23
Hillah	—	3	3	1	3	4	—	3	3	10
Kerbela	1	2	3	1	2	3	—	4	4	10
Kut-el-Amarah	—	—	—	—	3	3	—	2	2	5
Amarah	—	1	1	—	—	—	—	3	3	4
Kirkuk	1	1	2	—	—	—	—	—	—	2
Whole country	12	210	222	17	322	339	30	286	326	887

No case has been observed in the districts of Diyala, Mosul and Sulaimaniyah.
The cases reported at the Royal Hospital, Baghdad, are usually imported cases.

With the exception of a paper by SINDERSON and MILLS (1923) on " Rectal papillomata in *Schistosoma haematobium* infestation," a note by SINDERSON (1930) on the " Anomaly pigmentation in schistosomiasis," and a demonstration of an X-ray photo, by NORMAN (1935), no further work has been published on urinary schistosomiasis in Iraq. MACHATTIE and CHADWICK (1932) and MACHATTIE, MILLS and CHADWICK (1933) have described *S. bovis*.

A perusal of the Iraq Health Service reports on bilharziasis was made possible ·for MACHATTIE by the courtesy of Major T. J. HALLINAN, C.B.E., and the following relative information has been collected.

In April, 1921, there was an outbreak of urinary schistosomiasis amongst the British troops at Kufa. There were twenty-four cases. As a result, the urine of all the men of the 2nd Royal Ulster Rifles was examined for ova. It was found that over 25 per cent. of the Royal Ulster Rifles were infected. This regiment was stationed at Hinaidi for some time and it was considered possible that they had there infected the snails. Both at Kufa and Hinaidi search was made for the molluscan vectors. In May, 1921, Col. A. E. HAMERTON at Hinaidi collected numerous mollusca including *Bulinus* sp., but none proved to be infected with schistosome cercariae. The shells were sent to Mr. ROBSON of the British Museum for identification, who made the following report :—

1. *Isidora contorta*. Mich. undoubtedly.
2. *Planorbis* sp., very certain.
3. *Melanopsis nodosa*. Fer.
4. *Isidora contorta*. Mich.
5. *Era (Petraeus) halapensis*. Pfr.

6. *Isidora contorta*. Mich.,
   an extreme form of this species.
7. *Fruticicola obstructa*. Fer.
8. *Lymnaea persica*.
9. *Isidora contorta*. Mich.

The files of the Health Directorate further show that in 1923 the Civil Surgeon at Kirkuk found that in the village of Jibara near Kifri, " The majority of the male inhabitants seem to be infected."

At Ramadi, in 1925, there were nineteen cases in January, and sixteen cases in February. At Rumaitha, in 1925, 15 per cent. of school children complained of haematuria. The school children at Daghara and Khidther were reported infected.

Dr. RAMSAY, the Civil Surgeon at Nasiriyah, in 1925, reported that the incidence of bilharziasis in school children in the Muntafiq Liwa was probably about 80 per cent. The total of antimony tartrate injections given at the Maude Memorial Hospital at Nasiriyah, during the year 1924, was 3,084.

The health reports for 1925 show that at a recent inspection the incidence among school children was as follows :—

Ramadi, 8·8 per cent.; Saqlawiyah, 8 per cent.; Felujah, 3 per cent.; Qubaisah, 1·5 per cent.; Hit, 1 per cent.

At Nasiriyah in 1926, RAMSAY examined the urines of all the school children in the Government school, Nasiriyah, and found that out of 239 boys, 143 were suffering from bilharziasis. Another thirty-eight had a trace of blood in their urine, but no ova. If these are included, the possible total infection would be as high as 75 to 78 per cent.

In 1932, the writers, while investigating *S. bovis*, discovered twelve cases of *S. haematobium* in children at the village of Bankoora. There was no trace of intestinal infection such as is recorded by FISHER (1934) from the Stanleyville district of the Belgian Congo, although eggs similar to those depicted by FISHER were found in urine.

In 1926, ten cases were diagnosed in the Beni Tamin tribes, 20 miles from Baghdad.

In February, 1932, 100 persons were treated for bilharziasis in the village of Khan Beni Said (18 miles from Baghdad).

The incidence of bilharziasis in the Dulaim Liwa in 1930 and 1931 is as follows (Health Service report to Royal Air Force) :—

	Cases seen at Dispensaries.		Months of Maximum Incidence.	
	1930.	1931.	1930.	1931.
Anah	61	3	May : 20	August : 8
Hit			October : 2	October : 4
Ramadi			July : 36 August : 56 September : 37	February : 44 March : 58 September : 55
Felujah			October : 8 September : 4	September : 8

The report continues, " The returns for the year 1931, however, do not include the month of December which can be added later if required."

From the reports of the Royal Air Force the following information is available regarding an outbreak among British troops at Hinaidi, in 1924 :—

" The occurrence of two cases of bilharziasis in the early months of the year gave prominence to the possibility of the occurrence of this disease in much larger numbers. Examination of the rivers, bathing pools and ditches in the Hinaidi region demonstrated the presence of numerous snails of the bilharzia transmitting species. Subsequent bacterial examination proved the snails to be infected. A large number of urine examinations of personnel were carried out and over forty cases of the disease were discovered.

D

Investigations indicated that infection was distributed by a bathing pool and possibly by the Diyala river ; no cases were found amongst personnel who bathed in the Tigris. Bathing was restricted to a pool supplied with chlorinated and filtered water. The pool was periodically emptied, cleaned and creosoted. No further cases occurred as a result of the measures undertaken. Cases were treated with antimony tartrate, a full course consisting of 30 grains being given intravenously. No cases of relapse occurred."*

The above information regarding the Hinaidi outbreak was kindly supplied to MacHattie by the Principal Medical Officer who remarks as follows : " It is a little mislead- ing in suggesting that the Diyala was the source, as I am positive that every patient had bathed in the pool and that several had not bathed in Diyala. Ever since, the water in the bathing pool has been treated with cresol and copper sulphate, and no more cases have occurred since 1924 in this command."

From what has been written it will be gathered that bilharziasis has a wide occurrence and high incidence in Iraq. The figures, however, give us only an infinitesimal idea of the real position, because the majority of cases do not report for treatment.

II.—Technique.

A collecting apparatus was devised as it is inadvisable to collect snails with the naked hand. This consisted of a ¼ inch iron ring (9 inches in diameter, forged in the bazaar), on to which was sewn a strong net made of string, the whole being mounted on a stout pole 4½ feet long. For collecting snails from the mud at the bottom of ditches the ring of the net was beaten out into a knife- like edge, so a netful of mud was easily scooped up. Then by waving the net about in the water, the mud was washed free and the residue examined for snails.

Transport in very hot weather is a difficult matter. Placing the snails between washed layers of sand in a cigarette-tin (Leiper's method) is the best for transport by air mail. The lowest mortality for car journey was obtained by placing them in clay chatties, which can be purchased in any bazaar in Iraq. The temperature of the water, even on the hottest day, is kept low by the cooling effect of evaporation from the surface of the chatty. Snails transported in this way generally adhere to the cool inner surface of the chatty just at, and above, the water level.

In the laboratory the snails were placed in jars of fresh river water which was changed daily. (Great care must be taken to ensure that the water is unpol- luted by oil discharged by boats, etc.: many snails were lost before this was realized). Lucerne leaves were supplied as food. In this way *Bulinus truncatus* can be kept successfully for months during the summer without special means of water aeration. For examination for cercariae the snails were tubed separately and placed in the sun.

The living carcariae were studied in horse serum on a slide (Archibald and Marshall, 1931) and neutral red was employed for *intra vitam* staining.

*Extract from *Report of the Health of the Royal Air Force for the Year 1924*, Chapter iv, paragraph 47 (VI.), p. 74.

PLATE I

FIGS. 1 AND 3.—The infected pool at the Zoba tribe.

FIG. 2.—Method of infection of man, near Baghdad.

FIG. 4.—The ditch which infected British troops in Hinaidi. The medical authorities have now drained it and it contains no living *Bulinus*.

FIG. 5.—Another view of the infected pool at the Zoba tribe; a woman has waded in to obtain the family's water supply.

FIG. 6.—Method of infection of water carriers in Baghdad.

PLATE II.
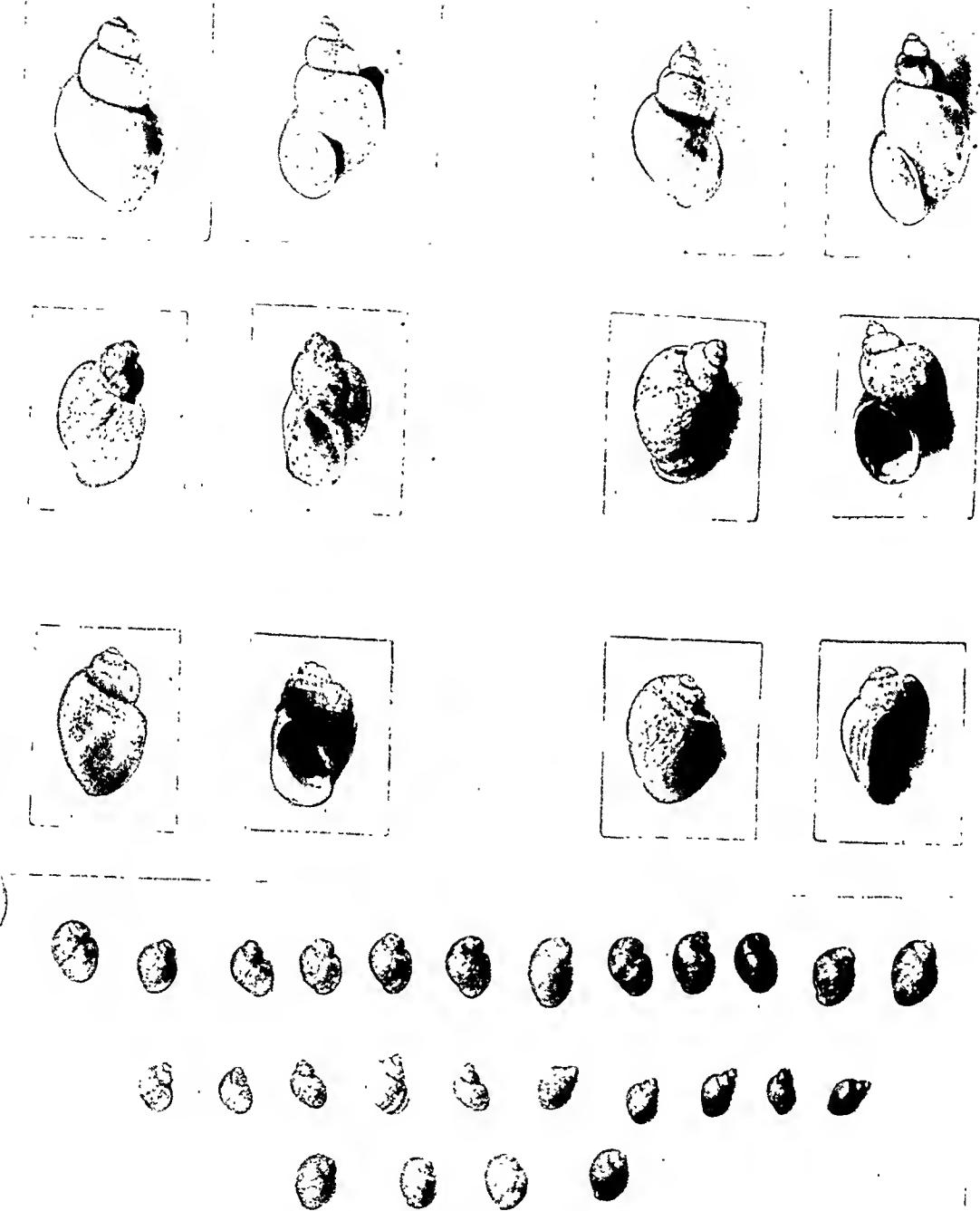
THE CARRIERS OF *S. haematobium* IN IRAQ.
Where this snail exists human bilharziasis is rampant.

For fixation and permanent preparations, Archibald and Marshall's hot lacto-phenol and gum-lacto-phenol techniques were employed.

III.—INVESTIGATION OF THE SNAIL HOST IN IRAQ.

a.—METHOD OF DISCOVERY.

A perusal of the health reports already mentioned shows that Diwaniyah, Nasiriyah and Basrah are centres of heavy schistosomiasis infection. These areas, however, have an intensive system of wide and deep irrigation canals and ditches, and it soon became evident that miles of water may be searched, and yet the infected snails may be overlooked. It was probably for this reason that Captain BOULENGER did not record living species of *Bulinus* in his investigation of the molluscan fauna. MACHATTIE, in carrying out the search for infected snails, disregarded these areas and searched for a small and intensive area of infection where the water supply was limited, and where new or freshly reported cases of schistosomiasis were known to occur.

An ideal site for such an investigation of the snail host was found at the settled Zoba tribe, some 20 miles in the desert from Baghdad. The tribe consisted of about 500 persons. Their sheep, camels and horses were entirely dependent for water on a single shallow canal which was replenished every 5 days from the river at Gurma, about 4 miles distant. Towards the end of the 5-day period, the tribesmen conserved the supply in the overflow pools shown in Plate I, Figs. 1, 3 and 5. A passing inquiry as to whether any of the tribesmen were passing blood in their urine, revealed that almost every man, woman and child in the tribe had such a history. A collection of urine from fourteen boys proved 100 per cent. strongly positive for ova. It was evident from the faeces surrounding the water pools that there was ample opportunity for infection of any snails which the pools might contain. The first search of the pools yielded eight specimens of *Bulinus truncatus* Aud.* in various stages of maturity. Two of these proved to be infected with mammalian schistosome cercariae which, in the authors' opinion, fulfilled the measurements and morphological characters of the cercaria of *S. haematobium.*

· These cercariae from *B. truncatus* obtained at the Zoba tribe, together with similar species from the same host from Baghdad and Amarah developed into *S. haematobium* in white mice (see pages 329 and 331).

*Specimens of the shells were sent to Mr. ROBSON who passed them on to Major M. CONNOLLY, who identified them as those of *Bulinus truncatus* Aud. It will be noted from Plate II and Major CONNOLLY's report on p. 332, that specimens of *Bulinus* found in Iraq differ considerably in shape, thus typical *contortus, dybowskii* and *innesi* types occur, also other forms showing a considerable degree of variation.

Table I shows the percentage infection of *B. truncatus* with this type of cercaria at the Zoba tribe during 1930-1931 :—

TABLE I.

Date.	Number Examined.	Number Positive.	Remarks.
1930—April 28th	A number	0	Little water in overflow pools
„ 30th	60	3	Only immature snails infected
June 2nd	30	1	
„ 8th	22	1	
„ 16th	35	3	
„ 19th	28	1	
„ 22nd	20	2	
„ 24th	79	2	
„ 26th	19	2	
„ 28th	27	1	
„ 30th	43	1	
July 4th	32	2	
„ 8th	186	6	
August 12th	26	2	
„ 31st	24	4	
September 8th	12	11	= 91·6 per cent.
„ 10th	22	9	
October 4th	16	12	
„ 12th	7	3	Infected but no emerged cercariae
„ 28th	10	1	
November 5th	12	1	
December 3rd	6	0	1 „ „ „
1931—January 5th	4	0	1 „ „ „
„ 12th	3	0	1 „ „ „
February 8th	3	0	
March 1st	12	0	2 „ „ „
„ 8th	9	1	1 „ „ „
April 2nd	8	1	1 „ „ „ ·
May 16th	14	1	

b.—INVESTIGATION OF *Bulinus truncatus* AUD.

Once the molluscan host was determined investigations were greatly facilitated. Nevertheless, 1,000 specimens were examined from various parts of the country before a positive snail was found. It would appear that *Bulinus truncatus* can only survive in very sluggish ditches and stagnant pools; too stagnant a pool, however, kills the snail. Despite intensive search, no positive snail was found in the Tigris, Euphrates or Diyala. Certainly the main streams are free from infection.

In summer, *B. truncatus* feeds on the green grass of the pool, when it is very prevalent in well demarcated areas. It was found that in one ditch about

1 mile long, there were areas where *Lymnaea* sp. existed exclusively, areas of mixed *Lymnaea* and *Bulinus*, and areas where only *Bulinus* occurred. Iraq has an extremely hot summer followed by a cold winter. In December, when the really cold weather commences, *Bulinus* sp. disappear as if by magic and are not greatly in evidence again in ditches until the end of May. Not only are the snails found with great difficulty after December, but observations made on 6,000 dissected specimens (MACHATTIE) show that there is a marked falling off in the percentage of infected individuals. It is also extremely probable that the emission of cercariae ceases altogether in the winter months, although the infection probably survives within the snail. A similar phenomenon is described for several species of cercariae (WESENBERG-LUND, 1934). There can be no doubt that in Iraq it is extremely dangerous to wash or wade in infected ditches or pools, from late June to early October, whilst from December to May the water of these places may be handled with comparative impunity, *i.e.*, there is a definite season of low infection possibility. The writers consider that the above described seasonal fluctuation in the degree of infestation of snails with larval *S. haematobium* is one of the most noteworthy points about this species in Iraq.

Remembering Iraq's exceedingly hot summers which coerce the fellaheen to bathe in the nearest water, and the cold weather when he has little use for water, it would almost seem as if nature had designed a special season periodicity to ensure the completion of the *haematobium* life cycle. From the point of view of snipe and duck shooters, this probably explains why so few enthusiastic shooters become infected. (The " open season " is from October to March.)

Although it is generally the mature, adult snails which show the largest percentage of *S. haematobium* cercariae infestation, it is also true that snails no larger than lentil seeds may show a heavy infestation and liberate cercariae in great numbers. It is, therefore, our experience that quite immature snails may be infected. This infestation has been noted in the *dybowskii*, *contortus* and *innesi* types of *B. truncatus*.

Infested snails are generally readily recognized as they are of a yellowish colour. Healthy *B. truncatus* are of brownish colour. It was noted that the shell of infested snails is much more friable and easily broken than the shell of healthy snails. Infested snails proved much more difficult to transport than healthy snails, but mature snails, whether of the *contortus*, *dybowskii* or *innesi* type of *B. truncatus* were always more delicate than immature snails which stood the motor journey well.

It may be relevant to mention here that *B. truncatus* and *L. tenera euphratica* Mous. are regarded by the marsh Arabs as edible.

c.—STUDIES OF AREAS OF INFECTION.

During the search for the snail host one or two interesting facts concerning infected areas came to light.

At the village of Madmudiyah situated between Baghdad and Hillah, the local health official had about fifty cases of schistosomiasis under spasmodic treatment. The village water supply was very limited, thus facilitating the search for the snail host. The only species present, however, were *Lymnaea* sp. 700 of which were examined with negative results. A closer inspection of incidence revealed that no new case had occurred during the previous $2\frac{1}{2}$ years. It was further learned that about 3 years previously, the irrigation department, for purposes of agriculture, had reorganised the water supply of the village, replacing a wide shallow ditch, which passed through the village and spread out in the centre of the town to form a pond where washing, etc. was carried out, by a narrow built canal through which the water flowed quickly. It is to be presumed that the snail host disappeared with the formation of the narrow swiftly flowing canal.

The position at Erbil proved to be very similar. The health reports showed that during February and March, of 1925, twenty-three cases of schistosomiasis occurred. A visit by MacHattie in 1930 proved that, although there were several cases reporting at the local hospital for treatment, no fresh case had occurred since 1924, when a wide and sluggish reservoir was built in and narrowed to a ferro-concrete tank, and water taps distributed in various streets. As has already been noted, it is probable that *B. truncatus* can only survive in very sluggish ditches and stagnant pools, although it cannot live in extremely stagnant water.

Occasional cases of urinary schistosomiasis reported at the Royal Hospital, Baghdad, but there appeared to be an idea that all of these cases were from the districts. A search of the Baghdad ditches and canals did not, therefore, hold much promise of success. However, *Bulinus* sp., apparently infected with *S. haematobium* ccreariae, were collected from the ditch running alongside the road from Baghdad to Hinaidi (via Alwiyah), the network of small canals which water the gardens of Alwiyah, and the Hinaidi road ditch. Investigation showed that the distribution of *Bulinus* snails was confined to definite parts of the ditches, these areas being scattered over a length of several miles. The finding of these suspicious looking cereariae in Baghdad started enquiries as to a possible source of infection. Living in and out of these ditches every day of their lives, are Arab water throwers, whose work is to throw the ditch water on the roads with a kerosene tin apparatus (Plate I, Fig. 6). Enquiry of these men showed that they were all passing blood in their urine. Some samples were taken and all showed *S. haematobium* eggs. Further enquiry of Alwiyah gardeners showed an 85 per cent. infection. A rough examination of fellaheen employed on cultivation along the road-side yielded twenty positive cases in 2 days.

The ditch running alongside the the road to Hinaidi constituted a serious public menace, since the water is drunk by passers-by travelling between Baghdad and Amarah (Plate I, Fig. 2). In 1933, large gangs of prisoners working in the vicinity were watered solely from this ditch. Travellers from Kut,

Amarah and the south have been frequently observed to stop just before entering Baghdad for a wash and clean up, using the water of the ditch for this purpose. The length and number of the ditches, however, made it difficult to find a heavy centre of snail infestation, but it became increasingly obvious as the investigation continued that Baghdad itself is a heavy centre of bilharziasis.

A localization of thousands of mature and immature *Bulinus* was found in a ditch at Hinaidi. In 1924, this ditch supplied the water for the swimming pool in which the Royal Air Force outbreak already referred to (page 321) occurred. The ditch supply to the pool was then cut off, and only water which had passed through the electrical station was used by the troops. None of these snails proved infested in 1930. The ditch was isolated due to the cutting down of the camp personnel, and its banks, previously lined with camps, were then deserted and there was no opportunity of snail infestation.

IV.—LABORATORY EXPERIMENTS.

a.—STUDY OF CERCARIAE.

It has already been stated that the cercariae from *B. truncatus* agreed with the morphological description of the cercariae of *S. haematobium*. Nevertheless, as individual workers have recorded some variation, it is considered advisable to give (Table II) the measurements obtained (by the method recommended by MANSON-BAHR and HAMILTON FAIRLEY, 1920).

TABLE II.

16 mm. lens	Body = 0·19591 mm.	8 mm. lens	Body = 0·1953 mm.
	Tail = 0·2457 „		Tail = 0·2324 „
8 „ „	Body = 0·1764 „	16 „ „	Body = 0·2483 „
	Tail = 0·2293 „		Tail = 0·2847 „
16 „ „	Body = 0·2230 „	8 „ „	Body = 0·2148 „
	Tail = 0·2600 „		Tail = 0·2652 „
16 „ „	Body = 0·1722 „	16 „ „	Body = 0·1527 „
	Tail = 0·2632 „		Tail = 0·2107 „
8 „ „	Body = 0·1616 „	16 „ „	Body = 0·2197 „
	Tail = 0·236 „		Tail = 0·292 „

Averages of 16 Readings.

Body	= 0·1928 mm.	Anterior to ventral sucker	= 0·129 mm.
Tail	= 0·239 „	Posterior to ventral sucker	= 0·044 „
Furcae	= 0·081 „	Diameter of ventral sucker	= 0·018 „
Diameter ventral suckers	= 0·018 „	Breadth of body	= 54 to 102
Total length body and tail	= 0·4308 „	Breadth of tail trunk	= 32 to 48

Attention was given to the behaviour of the cercariae as recent investigations (BARLOW, 1935) suggest that this study may lead to new prophylactic measures.

The cercariae were found to emerge from the snail, both in wild and laboratory infections, at a definite time of the day, *i.e.*, between 10.30 and 11.30 a.m. Even if the infected snails are kept in the dark, there is the same definite periodicity in the liberation of cercariae. In the laboratory at a temperature of 94 to 96° F. the cercariae showed loss of activity and sedimented after approximately 24 hours. In the autumn their activity is much reduced, but the life of the cercariae is longer by about 10 hours. In the hottest part of the year in the wild, the cercariae probably live less than 24 hours. Comparison of our notes for October with those of July, August and September shows that in the colder weather comparatively few cercariae are liberated. In the winter months, as no cercariae escape from snails, infection can only be proved by dissection.

Usually the maximum concentration of the cercariae is just below the surface of the water. Every now and then there is active movement to the actual surface. The remainder are fairly widely distributed throughout the whole column of water. The cercariae hang head downwards, and swim slowly to maintain their position in the water. Suddenly active movement takes place with upward progression, tail first, usually in a vertical direction, but occasionally somewhat laterally. Movement stops suddenly and the cercariae once again hang vertically. Although progress is never made with the tail downwards, it is sometimes held in a somewhat lateral position when swimming. In some cases the cercariae attach themselves to the wall of the tube by the ventral sucker, and may at intervals show violent lashing of the tail without progressive movement. At such times the tail may hang downwards in the water. It was noted that if tubes were brought from the darkness into light, most of the cercariae were attached to the sides of the tube in this manner, but after 3 to 5 minutes in the light great activity was noticeable. Those hanging vertically in the water, actively progressed towards the surface. Those attached showed active movement of the tail, and rapidly became detached from the wall of the tube and suspended throughout the water. Although the cercariae obviously showed some type of response to light, they displayed no positive or negative phototropism when a horizontal beam was passed through the water. A tube rich in living cercariae was surrounded by black paper and left for $\frac{1}{2}$ hour. Then a small window was cut in the paper and the tube was placed in the light for 1 hour. This experiment was repeated at 9 a.m., 12 a.m., and 4 p.m., but on no occasion was there any migration of cercariae to or away from the region of the window.

b.—INFECTION OF EXPERIMENTAL ANIMALS WITH WILD CERCARIAE.

It has already been noted that the *Bulinus truncatus* from the Zoba tribe showed a very high percentage of infection with the cercariae of *S. haematobium*. (See Table I.) The cercariae for these experiments were obtained from snails from this district. *B. truncatus* emitting cercariae were placed in distilled water.

A number of infected snails were used to ensure a constant supply of material as the emission of cercariae is somewhat irregular (see Table III). Guineapigs and mice were exposed to infection by three methods : (1) shaving the abdomen (subsequently washing with distilled water) and then securing the animal in a dish of distilled water containing active cercariae ; (2) immersing the tail of the mice in a tube of distilled water containing active cercariae ; (3) allowing the animals to drink from, and bathe in distilled water containing active cercariae. During the infection the laboratory temperature varied between 89° and 96° F.

Experiments.

White Mouse 1.

    6.6.30.—Mouse exposed to cercarial action by Method 1.

    7.6.30   ,,   ,,   ,,   ,,   ,,   ,,   ,,   ,, for ½ hour.

    11.6.30   ,,   ,,   ,,   ,,   ,,   ,,   ,,   ,, for 1 hour.

White Mouse 2.

    Tail of mouse immersed for 10 minutes in tube containing very numerous cercariae.

    The portion of the tail immersed in the infected water was markedly congested 20 minutes later.

    Mouse allowed to drink and walk about in dish containing very numerous cercariae, for 3 hours.

    After about 20 minutes' walking about in the water, the mouse sat up on its haunches and vigorously rubbed the forefeet together and against the mouth, as if trying to rub away some irritating bodies.

    14.6.30.—Mouse exposed to infection by Method 1, for 1½ hours.

Guineapig 1.

    8.6.30.—Guineapig exposed to infection by Method 1, for 40 minutes.

    About 12 minutes after immersion the animal became restive and struggled at intervals, possibly due to the irritating action of the cercariae. 30 minutes after exposure several pipettes of water near the animal's abdomen were examined. No active complete cercariae were observed, but several shed tails were in evidence. The animal appeared a little indisposed next day at 9 a.m., but recovered later.

    12.6.30.—Guineapig exposed to infection by Method 1, for 40 minutes.

Guineapig 2.

    10.6.30.—Guineapig exposed to infection by Method 1, for 25 minutes. The water was examined under a dissection microscope and showed a number of tails. Guineapig was noted to be indisposed at 8 p.m.

    15.6.30.—Guineapig exposed to infection by Method 1, for 40 minutes. The livers of infected snails were dissected out and added to the infested water.

    On 5.7.30 Guineapig 1, exposed to infection on 8.6.30 and 12.6.30, appeared thinner, otherwise the animals appeared healthy and the mice were lively.

    On 8.8.30 Mouse 1 appeared definitely sick, off its feed and sat huddled and shivering in a corner of its cage. Teased preparations of faeces showed no trace of eggs. It died on the night of 9.8.30 and was dissected on 10.8.30, *i.e.*, 64 days after exposure to infection. The abdomen was opened and the visible mesenteric blood vessels were searched under a dissection microscope. One worm could be seen moving in a large mesenteric vessel near the liver. The movements were distinctly active and showed progression by means of the suckers and also by distension of the body forming circular bulbous swellings in the portion of the worm posterior to the ventral sucker. This worm on removal was found paired and was the only one visible in the intact vessels.

    The liver was removed whole below the entrance of the portal vessels and transferred, to saline in a petri dish. Gentle washing produced five more worms, unpaired. The liver, lungs, spleen, kidneys and lower bowel and bladder were removed for section. The worms were identified as *S. haematobium.*

    On 4.9.30 Mouse 2 was dissected with negative results.

    On 5.9.30 Guineapigs 1 and 2 were dissected with entirely negative results.

TABLE III.

Date.	Time.	Snails.	Liberation of Cercariae.	Remarks.
1930 June 5	5 p.m.	3 mature snails from Zoba isolated	A few cercariae liberated	Snails placed in distilled water
6	10–12 a.m.	,, ,,	Enormous number liberated	,, ,,
7 7 7 7	10 a.m. 10.30 a.m. 6 p.m. 10 p.m.	Snails died Another 6 snails (1–6) isolated	Enormous number of cercariae liberated A few cercariae present in tube No increase in cercariae	Laboratory temperature, 96° F. Fresh distilled water had been added
8 8 8	8.30 a.m. 10.45 a.m. 5.30 p.m.		No liberation Snails 1, 2 and 3 liberated enormous numbers	Fresh water added to all 6 tubes
9	10.30 a.m.		Snails 1–3 again liberated large numbers of cercariae. Snails 4, 5 and 6 negative	
10 10	10.30 a.m. 6 p.m.	12 snails (1A–12A) tubed	Enormous numbers liberated by Snails 1, 2, 3	Laboratory temperature, 95° F.
11	10.30 a.m. 11.30 a.m.		Numerous cercariae shed by Snails 2A, 5A & 12A	Laboratory temperature, 94° F.
12	10.30 a.m. 11.30 a.m.		Numerous cercariae shed by Snails 2A, 5A & 12A	Fresh water added to tubes
13	10 p.m.	6 mature snails (1B–6B) tubed		
14	10.30 a.m. 11.30 a.m.		Numerous cercariae shed from Snails 3B & 4B	Laboratory temperature, 90° F.
15			Four of the snails shed cercariae	Laboratory temperature, 89° F. Temperature of water in tubes, 82° F.

c.—INFECTION OF *B. truncatus* WITH *S. haematobium* EGGS DERIVED FROM HUMAN URINE.

About six hundred specimens of *B. truncatus* were collected from Hinaidi in June, 1930. One hundred specimens were dissected but no trace of furco-cercous cercariae was found. The remaining snails were kept for two months in the laboratory, and a further fifty specimens dissected with negative results. All the shells showed the normal brown colour. By about the middle of August, 1930, we had at our disposal about 200 *B. truncatus* which could reasonably be supposed to be free from infection with furcocercous cercariae. On 15.8.30, fifty snails* were placed in two jars (Jars A and B). Human urine heavily infected with the eggs of *S. haematobium* was added to both jars. A month later (17.9.30) it was evident by examining the snails with the naked eye that the majority were of a yellowish colour, but several still retained their brown colour. On 17.9.30, one of the yellow coloured snails was removed from Jar A and tubed. In about half an hour large numbers of cercariae had emerged. Later all the light coloured snails proved to be infected. In this way between 17th and 25th September, cercariae were obtained from thirty-four snails. Eight of these snails died between 18th September and 2nd October. From this it will be noted that the average life of a snail infected in a laboratory is about 1 month to 6 weeks. Tubed snails only survive from 10 to 12 days at a temperature of 96° to 98° F.

It appears that cold greatly retards or prohibits development of the eggs or larvae of *S. haematobium* for in three similar infection experiments, carried out in the winters 1931-1932, we found it impossible to infect the snails. This supported our view regarding the seasonal fluctuation of *S. haematobium* in Iraq (see page 325).

d.—INFECTION OF WHITE MICE WITH CERCARIAE FROM EXPERIMENTALLY INFECTED SNAILS.

White mice only were subjected to the action of the experimentally reared cercariae, as the guineapigs had proved resistant to infection with wild cercariae. Methods similar to those employed with the wild cercariae gave the same satisfactory results. Eight females and twenty-two males of *S. haematobium* were recovered at postmortem examination. In no case was there a definitely shaped egg in the uterus.

V.—NOTES ON OTHER MOLLUSCS FROM IRAQ.

In view of the lack of success experienced by previous investigators in Iraq in attempting to trace the life cycle of *S. haematobium*, it was decided early

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*These snails laid egg groups varying from seventeen to twenty-two eggs from 20th to 21st August onwards. The average water temperature was 72° F.

in this investigation to follow the suggestion of BOULENGER (1919) and examine some of the other Gastropoda of the country. *Lymnaea* sp. arc by far the most prevalent gastropods in all parts of central and southern Iraq, particularly *L. tenera euphratica* Mous. The term " *euphratica* " is rather a misnomer since thc snail occurs with equal prevalency in both the Tigris and Euphrates areas.

From various districts, as follows, 11,300 specimens of *L. tenera euphratica* were dissected with negative results :—Amarah, 6,000 ; Baghdad, 2,500 ; Baqubah, 750 ; Zoba, 500 ; Hillah, 500 ; Basrah, 300 ; Diwaniyah, 250 ; Khanaquin, 500.

Although urinary schistosomiasis is prevalent at Amarah, a week's search during midsummer soon made it evident that *B. truncatus,* so prevalent at Baghdad, is at Amarah comparatively rare. A search at Basrah on three occasions failed to find a single *Bulinus* snail. *L. tenera euphratica* and *L. canalifera* Mous. (= *L. peregra* race *canalifera* A. and P.) only existed in the ditches and canals of several infected tribes. Accordingly an intensive dissection of *Lymnaea* was carried out at Amarah in 1930, 1931, 1932. Although the snails proved to be entirely negative for *S. haematobium* cercariae, *L. tenera euphratica* harboured the cercariae of *S. turkestanicum* and two other types of non-mammalian furco-cercous cercariae. (MACHATTIE, 1935). Apart from those in these rice ficlds at Amarah, no furcocercous cercariae were ever found by MACHATTIE in Iraq in *Lymnaea* sp., with the exception of a single snail from Hindiyah, which was infested with a non-mammalian type of larva.

Planorbis philippii Monts. was found to be prcvalcnt in the crecks at Basrah and fairly common in the ditches at Amarah. 750 specimens were disscctcd at Basrah and 150 at Amarah, but no furcocercous cercariae were encountered. An intensive search extending over 3 years has shown a complete absence of *Planorbis* sp. in Baghdad Liwa.

The only other two snails found to be prevalent in bilharzia infested areas are *Melanoides tuberculata* Mull. and *Melanopsis nodosa* Fer. From the latter, at Diwaniyah and Baqubah, non-mammalian furcocercous cercariae have been repeatedly recovered.

Major M. CONNOLLY's report on the collection of Iraq shells is as follows :—

" Report on Mesopotamia Mollusca submitted for identification by the Imperial Bureau of Agricultural Parasitology, St. Albans.

Note.—These determinations are based on papers by MOUSSON (1874) and ANNANDALE and PRASHAD (1919-20).

(*a*) Fresh water species.

    1. *Lymnaea euphratica* Mouss. (*L. tenera* race *euphratica* A. & P.).
    2. *Lymnaea canalifera* Mouss. (*L. peregra* race *canalifera* A. & P.).
    The large shells are this species, and probably also the very small ones with them are young of the same, though they might be *L. cor.,* A. & P.
    3. *Planorbis philippii* Monts., agreeing with examples from Tartous sent me by PALLARY ; they ought to be *Plan. intermixtus* Mouss., but do not appear to quite agree with either figures or description.

4. *Bulinus truncatus* Aud. (*contortus* Mich., *brocchii* Ehrn, etc.), a very variable sp[ecies] to which all the Mesopotamian forms are referable. I agree with ANNANDALE (*Ind. Jo[urnal] of Med. Research*, x, 1922, p. 484), and BAYLIS (*Ann. Trop. Med.*, xxv, 1931, p. 37[ ]) placing all later names in the synonym of the oldest, namely, *truncatus*.

5. *Melanoides tuberculata* Mull.

6. *Melanopsis nodosa* Fer.

7. *Neritina* of *bellardii* Mouss., a single specimen in rather poor condition.

8. *Neritina* of *jordani* Sow., var. *aberrans* Dautz., two small specimens which I c[annot] guarantee ; I do not think they have ever been accused of spreading disease, so are not v[orth] the hours of research that would be necessary to determine any more exact name tha[n has] been applied to them.

9. *Corbicula fluminalis* Mull., a bivalve of no medical interest as a disease carrier.

(*b*) Land species, which cannot have any bearing on the above matter.

10. *Helix* (*Helicogena*) *figulina* Parr.
11. *Helicella* species ? (a singleton).

12. *Petraeus aleppensis* Pfr.

13. *Fruticicola obstructa* Fer.

(*c*) A miscellaneous lot of beach-rolled marine shells, many immature, all in [poor] condition, and with no bearing on medical requirements ; not worth identifying, u[nless] there is a special request for it."

VI.—SUMMARY.

1. The prevalence of human schistosomiasis in Iraq is reviewed and [dis]cussed. It is shown to be of wide occurrence and high incidence.

2. The life history of *Schistosoma haematobium* in Iraq is described for [the] first time.

3. *Bulinus truncatus* Aud. serves as the molluscan intermediate host. [The] snail can apparently only survive in ditches and stagnant pools, and is ab[sent] from swifter streams.

4. Baghdad, in addition to other areas, is shown to be a centre of h[eavy] infection.

5. One of the most significant points regarding the cycle of *S. haemato[bium]* in Iraq, is the occurrence of a definite season of high infection possib[ility] (June to October) ; and a definite season of low infection possibility (Decem[ber] to May).

6. Laboratory experiments prove that the wild cercariae from *B. trun[catus]* will develop in white mice. Cercariae derived from snails, experimen[tally] infected by *S. haematobium* ova from human urine, will also develop in w[hite] mice. Guineapigs proved resistant to infection.

7. Numerous other species of snails, including 11,300 *Lymnaea te[rvia]* *euphratica* Mous., from infected areas, were examined for cercariae of *S. haem[ato]* *bium*. All proved negative for this species although infected with o[ther] cercariae.

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BILHARZIAL APPENDICITIS IN
SCHISTOSOMA HAEMATOBIUM INFESTATIONS.
A PRELIMINARY REPORT.*

BY

A. C. LOVETT-CAMPBELL,

Medical Officer, Colonial Medical Service, West Africa,

AND

A. W. ROSE,

Pathologist, Colonial Medical Service, West Africa.

The purpose of this paper is threefold ; firstly, to record the frequency with which *Schistosoma haematobium* ova and grosser bilharzial lesions implicated a series of thirty appendices, removed at operation from Africans inhabiting a hyperendemic area of Northern Nigeria ; secondly, to discuss certain points in the symptomatology of these cases ; and lastly, to describe serially and in some detail the histological changes evident in what are judged to be early, intermediate and late phases of tissue reaction to local ova infiltration within the appendix.

A series of photomicrographs illustrates these changes, and serves to amplify the descriptive histopathology of the condition.

In preparing this paper, access to selective literature disclosed the thoroughness with which regional distribution of schistosome ova throughout abdominal viscera had previously been investigated by early workers, notably FAIRLEY (1919), MADDEN (1922), and DEW (1923).

While thus disclaiming precedence for our observations, we desire to stress certain facts emerging from our recent investigations, which hitherto appear to have received scarcely adequate mention. For example, in our

serics of cases, the notable frequency of appendicial lesions* arising exclusively from *S. haematobium* infestations, deserves particular emphasis, inasmuch as only scant reference to isolated instances of this nature appear in most up-to-date text-books.

Only four patients had unmistakable appendicular colic and, in each of these, the implicated appendices had characteristic bilharzial lesions of macro-scopic degree serving to demonstrate, beyond all reasonable doubt, that occasion-ally acute appendicitis—clinically indistinguishable from its more familiar surgical counterparts—can, and does in fact, arise from uncomplicated *S. haematobium* infestations.

Thirdly, in contrast with the above, a majority group, comprising sixteen individuals with ova-infested appendices, and fifteen individuals with negative appendices, gave histories, in which the predominating symptoms were diffuse abdominal colic, associated with mucoid diarrhoea. Indeed, the predominance of these symptoms suggested extensive colonic involvement, while accompanying appendicial lesions—present in just over half the number—being of minor degree, were, clinically, either masked or actually the cause of no local symptoms. That the prevailing lesions in this group were colonic, lacks pathological con-firmation, as none of our cases came to autopsy. But it has long been observed that mucoid diarrhoea occurs in association with *S. haematobium* infections, and that terminal spined ova occasionally appear in the stools of these patients, while autopsies confirm the frequency of colonic and rectal lesions.

Our first patient was operated upon for acute appendicitis.

Case History.

Early in March, 1935, an African male, aged 30, was admitted to hospital with a history of recurring, low abdominal pain, diarrhoea and vomiting, of three years duration. Attacks were frequent, usually occurring about every 10 days, and subsiding gradually, after 3 or 4 days. Vomiting always ceased early.

Symptoms almost similar to the above were actually witnessed while the patient came under observation in hospital. Closer examination disclosed definite tenderness, more or less constant, over the appendix area, unaccompanied by local rigidity. On digital pressure, marked gurgling of the caecum could be elicited. Faeces and urine were free from ova. Red blood cells were present in both. Cysts of *Entamoeba histolytica* were also detected in the faeces.

Classified as a dysenteric, this patient was given one week's treatment with emetine and yatren.

As no noticeable abatement of symptoms resulted, appendicectomy was performed. At operation a large free appendix was removed. Its extremity was bulbous and tense. The serous coat was injected. Slender, easily divided bands encircled a distended caecum, and ballooned the distal sacculations of the ascending colon.

The patient made an uneventful recovery, with immediate and complete cessation of symptoms.

The pathological comments read as follows :—" The appendix shows a condition of subacute inflammation, and infiltration with round cells and eosinophils, associated with the presence of schistosome ova in the submucosa. Curiously enough, these appear to be *S. haematobium*, rather than *S. mansoni*. The condition is an interesting one."

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* The term " lesion " is employed here in a wide sense to include the deposit of ova with or without tissue reaction resulting therefrom.

During the subsequent 6 months, thirty-four more appendicectomies were carried out. Each case was investigated clinically, with a view to establishing a possible relationship between symptoms and the pathological changes encountered.

All patients at the time of admission to hospital were excreting *haematobium* ova in their urines. Four appendices had gross macroscopic lesions. Together with the histories given, these four specimens provide convincing proof that acute appendicitis, clinically indistinguishable from its pyogenic forms, occasionally arises in bilharziasis. This is contrary to the view of BARSOUM (1934) that bilharziasis does not cause or predispose to appendicitis.

Thirty other cases in the series, including sixteen with infested appendices, manifested characteristic dysenteric symptoms—abdominal colic and profuse mucoid diarrhoea.

The constancy of these symptoms in so many cases, irrespective of whether appendicular infestation with ova was proved to exist or not, favours the conclusion that their seat of origin is the colon, not the appendix ; that only in the presence of gross bilharzial lesions of the appendix, do symptoms referable to that organ overshadow associated colonic symptoms.

As regards the discovery of ova within the lumen of the appendix, it is conceivable in certain instances that ova may gravitate accidentally there from the caecum, and thus come to lie between the glandular crypts.

Below, an account is given of four cases, previously mentioned as having gross lesions of the appendix.

Case 1.—Male aged 25.

Clinical.—Symptoms commenced 9 years ago, since when he experienced four severe bouts of abdominal pain. Vomiting preceded each attack, and pain was relieved by lying face downwards. The onset was always gradual. The pain attained great severity, and subsided at the end of 2 days. It was always most marked over the appendix region, the patient adding that in this area the abdomen became " like a stick " (rigidity). Diarrhoea and looseness of the bowels were frequent, but lately he has tended to be constipated.

Pathological.—The appendix was distorted and kinked 2 cm. from the tip by a fibrous tumour, $1\cdot4 \times 1 \times 1$ cm. in size, which occupied half of the circumference of the appendicular wall on the side remote from the mesocolic attachment. The mucosa was intact but pressed upon by the tumour, the lumen being distorted and crescentic in shape, with the concavity towards the tumour.

The mucosa showed the presence of congestion and catarrh, and the lumen contained a plug of amorphous matter coated with a layer of mucus, desquamated epithelial cells, and erythrocytes.

There was intense infiltration of the mucosa with round cells and eosinophil polymorphs.

The tumour was composed of chronic inflammatory reaction surrounding numerous ova of *S. haematobium*, which had apparently been deposited in the submucosa, at a point remote from the mesocolic attachment. The circular muscle coat was thickened, and showed hyaline change, and had been ruptured. The ruptured ends were plainly seen, separated by the inflammatory mass. Three-quarters of the tumour lay peripheral to the muscle coat. The surface of the tumour was smooth, except for the presence of minute greyish tubercles 1 to 2 mm. in size, which were very slightly raised above the surface. The structure of the tumour varied slightly in different parts ; broadly speaking it tended to

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be more cellular within the muscle coats, *i.e.*, in the usual situation of the submucosa, and more fibrous outside these coats at the periphery of the tumour. This favoured the inference that the latter was of longer standing than the former. The serous coat was seen stretched out over the surface of the tumour for a short distance, and then merged into it.

The structure of the inflammatory mass was as follows :—

Whorls of fibrous tissue surrounding single or multiple ova were embedded in a matrix of cellular and vascular granulation tissue, abundantly infiltrated with wandering cells, round cells, and eosinophil polymorphs. The fibrous whorls differed from each other, and the difference was believed to be due to their respective ages.

Five types were seen :—

(1) A dense concentration of eosinophils and wandering cells around the ovum, with outside this a layer of cells resembling fibroblasts.

(2) Similar to (1) but the eosinophils had undergone degenerative change although the nuclei were still visible. The fibroblasts were ranged radially to the central mass like the spokes of a wheel, and a concentric wall of fibrosis limited the structure.

(3) The granular débris was no longer apparent, but foreign body giant cells were commonly found. Phagocytosis of the ova could be seen.

(4) Similar to the above but very little remained of the ova, and fibrosis was more marked.

(5) A concentric fibrous scar alone remained.

Case 2. *Male aged* 20.

Clinical.—Abdominal trouble commenced 6 years ago, characterized by colicky pain and mucoid diarrhoea. Mild attacks occurred at intervals of 4 months, were gradual in onset, and continued for at least a day. No vomiting occurred. No early haematuria had been noticed.

Pathological.—Two cm. from the base of the appendix there was an irregular swelling 2 × 1 cm. in size formed by a localized increase in the appendicular wall. The peritoneal surface was smooth and glistening, but minute greyish tubercles could be seen in the substance of the tumour. The tip of the appendix was also distorted by a small fleshy beaklike tumour showing the presence of tubercles. A smaller pedunculated mass was found at the junction of the middle and distal thirds and resembled a smooth wart. The lumen was filled with a plug of mucus and catarrhal epithelial cells. The mucosa was abundantly infiltrated with round cells and eosinophil polymorphs. The germinal centres of the lymph nodes were prominent. The longitudinal muscle coat and subserous coats were much thickened by the presence of granulation tissue heavily infiltrated with eosinophilic polymorphs. *S. haematobium* ova were present. The tissue was vascular. Here and there were pseudo-tubercles of early type. The circular muscle coat was split by pseudo-tuberculous reaction. The submucosa also contained ova, and here the reaction was of the giant cell type with phagocytosis of the ova. The subserous coat was thickened to such an extent that it equalled in breadth the rest of the appendicular wall.

Case 3. *Male aged* 16.

Clinical.—Abdominal trouble commenced 3 years ago with severe attacks of colic that occurred every 3 months. Vomiting preceded their onset while during attacks pronounced mucoid diarrhoea was a feature. Pain commenced in the epigastrium and spread to the right iliac fossa. Relief was obtained by pressure over this area. Haematuria, present from childhood, still recurred, usually at intervals of 3 months.

Pathological.—The appendix proper was fairly normal in appearance, but present at its distal extremity was a dense band of adhesions about 2 cm. long containing a leash of vessels. This band of tissue connected the tip of the appendix with a solid oval tumour 1½ × 1 cm. in size. The adhesions were twisted on themselves. The surface of the tumour was nodular owing to the presence of large numbers of minute greyish tubercle-like elevations.

(a) The appendix proper.—The lumen contained erythrocytes and catarrhal epithelial cells. The mucosa was congested and infiltrated with round cells and eosinophil polymorphs
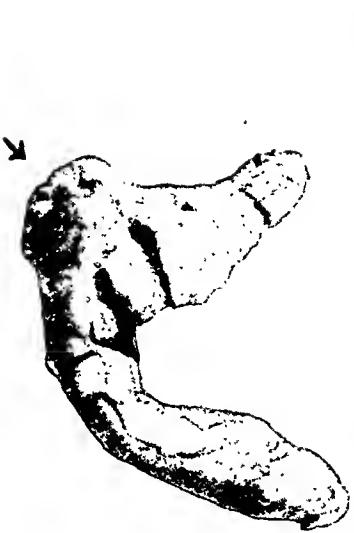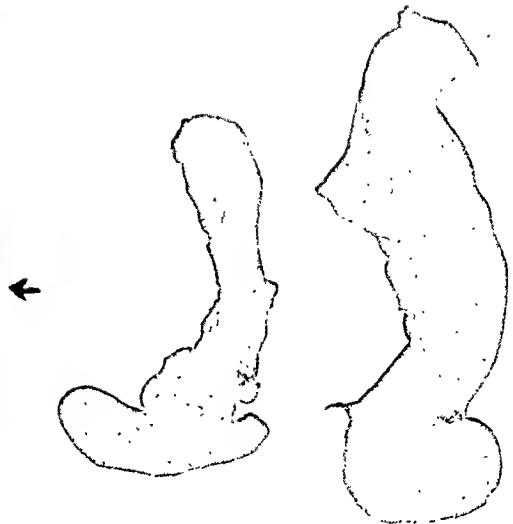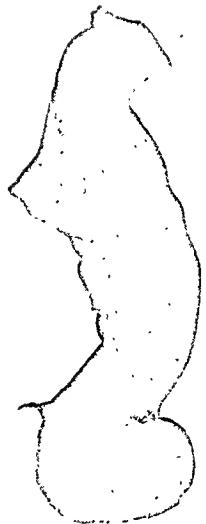
FIG. 1. FIG. 2. FIG. 3. FIG. 4.
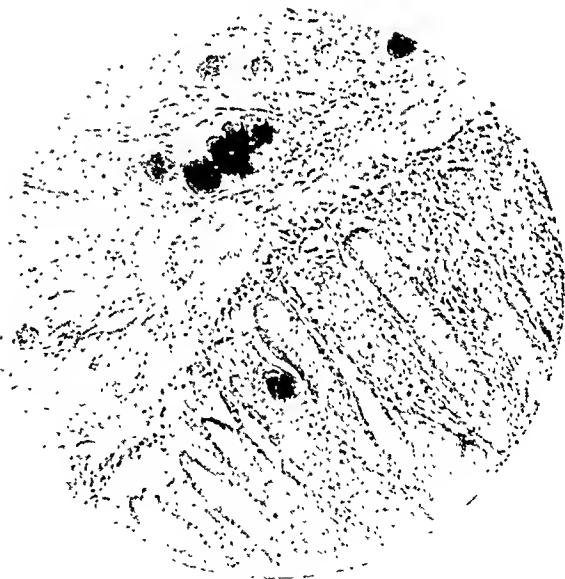
FIG. 5. FIG. 6.

FIGS. 1-4.—Appendices from Cases 1-4.

FIG. 5.—Calcified ova in the submucosa. Note in this case the absence of specific tissue reaction. × 27.

FIG. 6.—Similar to Fig. 5, but showing ovum lying between the glandular crypts. × 93.
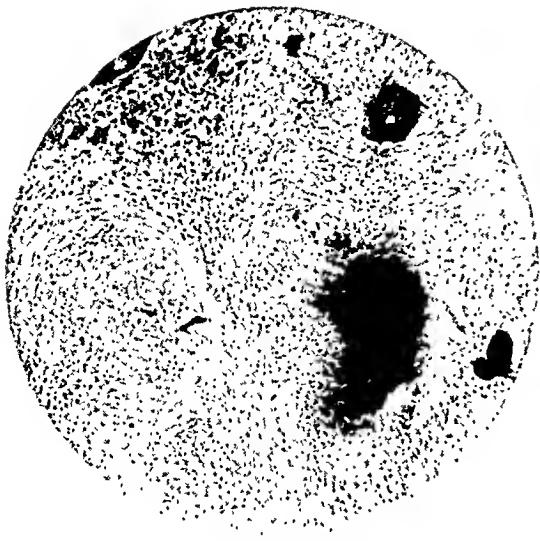
FIG. 7.
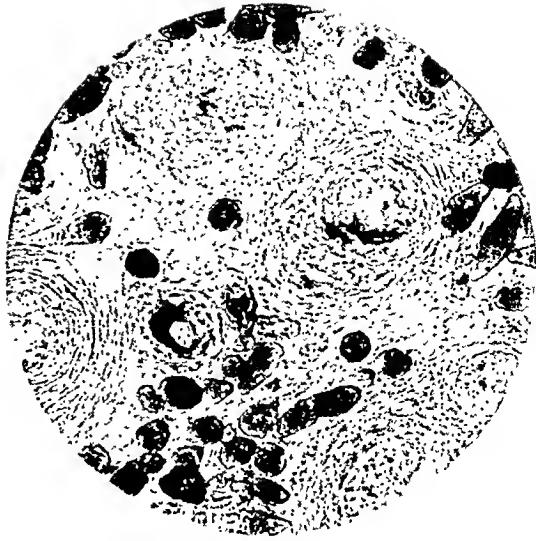
FIG. 8.
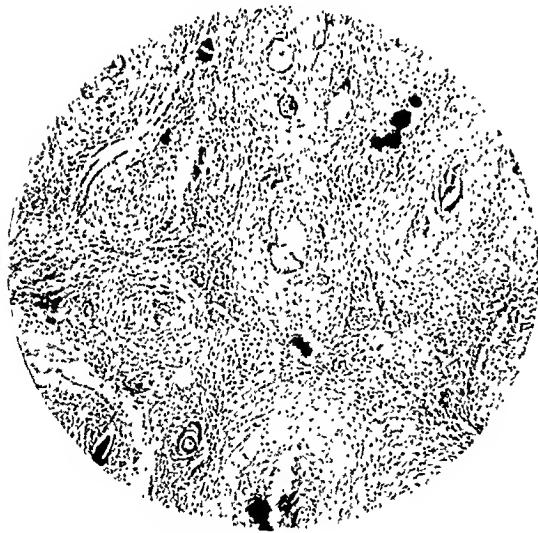
FIG. 9.
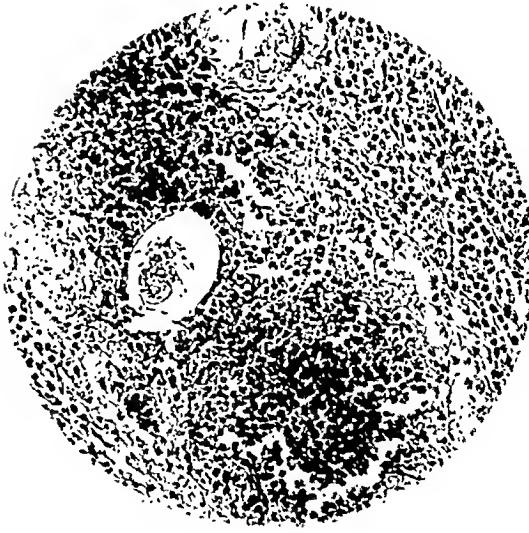
FIG. 10

FIG. 7.—Pseudo-tuberculous granulation tissue.   Note the presence of several ova.   × 93.

FIG. 8.—Concentric whorls of fibrosis in relation to very numerous degenerate ova.   × 93.

FIG. 9.—Pseudo-tuberculous tissue demonstrating the presence of cellular infiltration, calcified ova, and multinucleate giant cells.   × 60.

THE EVOLUTION OF THE PSEUDO-TUBERCLE (FIGS. 10-14).

FIG. 10.—Ovum surrounded by concentration of eosinophil polymorph cells.   × 175.
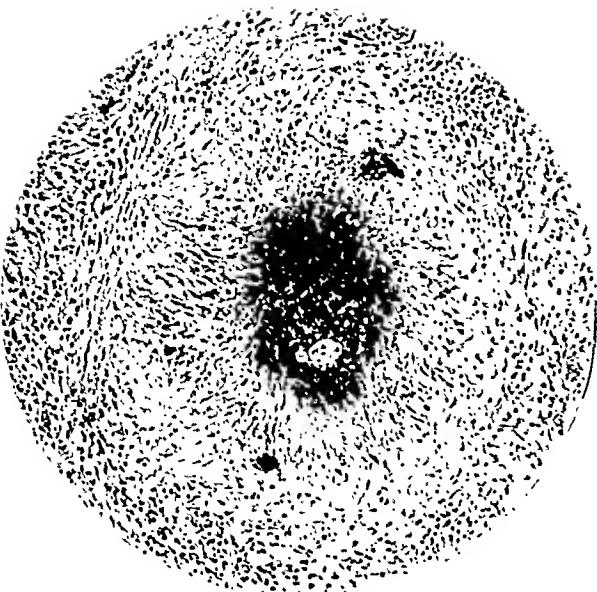
FIG. 11.
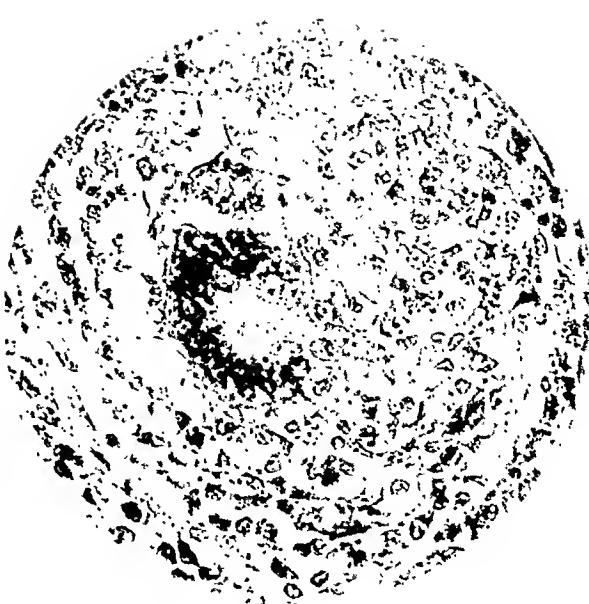
FIG. 12.
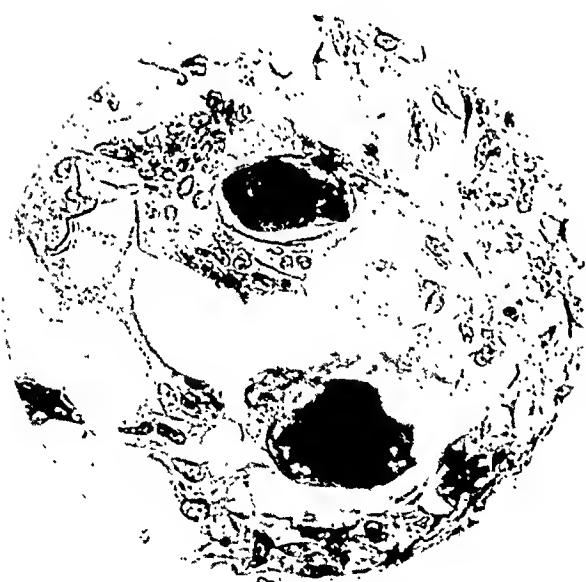
FIG. 13.

FIG. 14.

FIG. 11.—Granular degeneration of cells around central ovum. Note the spindle cells arranged radially around the central mass. × 95.

FIG. 12.—Multinucleate giant cell. × 360.

FIG. 13.—Phagocytosis of calcified ovum by multinucleate giant cell. × 360.

FIG. 14.—Fibrous scar. × 360.

and contained a few *S. haematobium* ova. The submucosa contained clumps of ova numbering 1 to 10 but there was no characteristic reaction. They were situated remote from the mesocolic attachment. The serous coat was thickened and infiltrated with ova.

(*b*) The tumour.—This consisted of a solid mass of pseudo-tubercles surrounding myriads of ova which were mainly calcified. The tubercles were of an advanced type although giant cells were commonly present.

(*c*) The adhesions.—These presented a similar picture to that shown by the tumour.

Case 4. Male aged 37.

Clinical.—Symptoms commenced 5 years ago with mid-abdominal colic. Attacks alternated with periods of freedom. He recollected four pronounced attacks in 5 years. Each continued for about 3 days. The pain commenced low down above the middle of the right inguinal ligament, and compelled him to lie still. There was no vomiting, but diarrhoea and the passage of blood and sometimes mucus in slight amount occurred. There had been haematuria in childhood.

Operation 14th Sept., 1935.—The appendix was difficult to locate, lying retrocaecally buried in adhesions. The caecum was fixed and immobile. The appendix was enlarged and tortuous. The serous coat was much injected.

Pathological.—The lumen contained a catarrhal plug. The mucous membrane was slightly thickened with marked cellular infiltration. The submucosa, also slightly thickened in parts, was infiltrated with round cells, eosinophils, and fibroblasts. *S. haematobium* ova were scattered about it singly and in pairs. The muscular coat appeared thickened. The serous coat was definitely thickened in parts and was very vascular. Fairly numerous ova were present in its substance. Many of the smaller vessels in the serous coat were cuffed with lymphocytes. The serous coat at one point was thickened to form a fleshy adhesion binding down the kinked tip of the appendix to the main body of the organ.

PATHOLOGICAL TECHNIQUE AND OBSERVATIONS.

The specimens numbering thirty-five in all were fixed in 10 per cent. formol-saline. A searching macroscopic and microscopic examination was carried out in each case, and particular attention was paid to the following points.

Macroscopic.—Measurements including length, average diameter, and thickness of the wall; abnormalities of conformation including kinking, nodules, or callosities; the presence of adhesions and congestion of the surface vessels; search of surface vessels and also the vessels of the mesoappendix for adult helminths. The appendix was subsequently opened up down its length, and the presence or absence of concretions, foreign bodies, adult helminths, blood and mucus recorded.

The mucosa was examined for evidence of hyperplasia, congestion, oedema, polypoid growths, and ulceration; the state of configuration and the patency of the lumen was observed.

Microscopic.—The organ was then examined microscopically as follows :—

(1) Scrapings taken from the mucous and submucous coats together with a portion of the contents of the lumen triturated in normal saline.

(2) Portions of the complete circumference of the appendicular wall at the base, centre and tip, finely divided and digested in 4 per cent. KOH at 56° C.

(3) Paraffin sections of the complete circumference of the appendicular wall at the level of the base, centre and tip. No serial section method was employed.

Any obvious macroscopic lesion was dealt with in a similar manner. In addition, in all cases failing to reveal ova by these methods, the entire remains of the appendix were digested and the deposit re-examined.

In this way twenty out of thirty-five specimens revealed the presence of *S. haematobium* ova (including four cases in which gross macroscopic lesions were present) and the number of ova present was found to vary considerably in each case. The number on the whole was greatest in those cases showing macroscopic lesions, but that this was not invariably so will be shown later. It was found, as one would expect, that when scanty the ova could be detected by digestive methods more readily than in paraffin sections and scrapings. No *S. mansoni* ova were ever encountered. The accompanying table summarizes the results.

Positive by different methods of examination.	
Scrapings, section, and digestion	13 (65 per cent.)
Section and digestion only	4 (20 ,,)
Digestion only	3 (15 ,,)
	Total 20 cases.
Negative for *S. haematobium* ova by all methods.	
	Total 15 cases.

It will be noted that the specimens can now be divided into two groups.

Group 1.—Twenty cases revealing the presence of *S. haematobium* ova, four of which showed gross macroscopic pathology directly due to the presence of the ova.

Group 2.—Fifteen cases showing no evidence of the presence of bilharzia ova.

In view of the fact that only one completely normal appendix was encountered, it will be convenient to contrast in tabular form, and discuss the findings in these two groups.

Pathology.	Group 1. 20 bilharzia positive cases.		Group 2. 15 bilharzia negative cases.	
	Present.	Absent.	Present.	Absent.
Abnormality of conformation	12	8	7	8
Congestion	12	8	10	5
Adhesions	9	11	5	10
Adult *Enterobius vermicularis*	11	9	7	8
Concretions	5	15	1	14
Eosinophilia	20	0	13	2
Catarrh	13	7	8	7

In assessing the value of the findings contrasted above it is necessary to bear in mind the difference in the total number of cases in the two groups, and the fact that the former group contains four cases in which the pathological findings could be shown to be directly due to bilharziasis. It then becomes apparent that almost as many cases in Group 2 show abnormalities of conformation, congestion, adhesions, catarrh, eosinophilia, etc., as those of Group 1. Concretions may be quoted as a possible exception to this rule, and in several cases they were multiple, of small size, and quartz-like in appearance, owing their origin, it may be, to inspissated mucus associated with catarrh of the mucosa. It is not conclusive, therefore, except where typical macroscopic lesions are present, that the abnormalities mentioned above are necessarily due to the presence of the ova. Attention is drawn to the large number of cases in both groups in which adult *Enterobius vermicularis* was found (eighteen cases).

Site of bilharzia ova.			
Submucosa	5 cases	Submucosa and Serous coat	1 case
Submucosa and mucosa	7 cases	All coats including muscular	4 cases
	Unknown 3 cases		

It will be seen that the ova were more commonly found in the submucous coat then elsewhere, but that no coat was exempt.

FAIRLEY draws attention to the rarity with which ova were found in the muscular coat of the intestine in his experimental work on monkeys, and it is of interest to note, therefore, that in our series, ova and inflammatory reaction were sometimes seen actually in the substance of both muscle coats separating the muscular fibres.

In all the cases showing macroscopic pathology, ova were demonstrable in the serous or subserous coats, and in only one case where these coats were invaded by ova was macroscopic pathology absent.

The ova were found singly or in clumps up to about twenty in number, and were usually situated at some point remote from the mesocolic attachment. The ova were usually found in the tissues, and not in the blood vessels. Occasionally they all appeared to be healthy, and the contained miracidia could be plainly seen ; but more often some, or all of them, were degenerate or calcified.

They were usually demonstrated throughout the length of the organ, and had no orderly arrangement, so that on section they might be cut in any plane. When in the mucosa they were always seen to lie between the glandular crypts, and in this situation the spine was usually directed towards the lumen.

The Number of Bilharzia Ova.

The number of ova appeared to vary in each case but no accurate count was made. The figures given are merely a rough estimate.

> Very numerous, 3 cases.   Numerous, 7 cases.
> Fairly numerous, 5 cases.   Scanty, 5 cases.

It is believed that the presence of gross pathology did not entirely depend on the number or concentration of ova present (although doubtless these are factors of some importance), and as evidence of this we find that cases showing very numerous ova did not always present gross pathological lesions ; furthermore, two of the cases showing gross lesions were not remarkable for the frequency with which ova were encountered.   · .

Tissue Reaction.

No reaction at all could be seen in seven cases harbouring ova.

In seven other cases there was some evidence of non-specific fibrosis, or young fibroblastic reaction in the region of the ova.

In the remaining six cases reaction was observed which, from a careful study of the sections, is believed to be typical of the condition under discussion. In two of these six cases, the lesions were judged to be early ones, and in the other cases all stages or only late stages were seen.

It has been possible collectively to build up what is believed to be the pathological process at work, and amply to confirm the earlier records of FAIRLEY and DEW. This process is illustrated step by step in the accompanying photomicrographs.

The earliest changes were found most characteristically in the serous and subserous coats, where in response to the deposition of ova a peculiar type of granulation tissue is laid down. The latter consists of a fibrous stroma abundantly infiltrated with polymorphonuclear eosinophils and a lesser number of round cells and wandering cells. Very numerous capillaries course through its substance. In consequence of this the serous coat becomes enormously thickened and may equal in breadth the remainder of the appendicular wall. There is great concentration of the eosinophils in the immediate vicinity of the ova, which at this stage are either quite healthy or only slightly degenerate. The capillaries may show lymphocytic " cuffing " and there may be hyperplasia of the endothelium of their walls.

In places the reaction may have progressed to the formation around the ova of a characteristic structure, the pseudo-tubercle.

The macroscopic appearances at this stage may consist of a localized thickening or bulging of the external surface of the appendix, or they may take the form of fleshy adhesions. The serous coat may be smooth except for the presence in its substance of slightly raised greyish nodules very similar in appearance and size to miliary tubercles. These are in reality pseudo-tubercles and are formed in the following manner.

The eosinophils which are concentrated immediately around the ova commence to degenerate. Very soon mononuclear cells, wandering cells, and fibroblasts range themselves around the degenerate central mass in a radial manner like the spokes of a wheel. Multinucleated giant cells, similar to those found in tuberculous tissues, make their appearance, and a confining wall of hard fibrous tissue is laid down at the extreme periphery.

By this time the central eosinophils have entirely degenerated, and have been converted into a granular mass with only a few pyknotic remains of the nuclei left to indicate its origin.

Probably the ova themselves are by now completely degenerate and possibly calcified. In a typical example the resemblance to a caseous tubercle may be striking. The giant cells appear to act as scavengers, and are often seen surrounding or engulfing the calcified remains of ova.

The cells of the central zone which are ranged round the central mass like the spokes of a wheel are mainly elongate, and correspond in position, and somewhat in appearance, to the endothelioid cells of the true tubercle. Occasionally they are seen giving off protoplasmic filaments which connect them with each other and the granular mass which they surround. Sometimes they are aggregated into a mass in which it is difficult to distinguish their individual outlines. It is tempting to believe that this is a step in the formation of the multinucleate giant cell which is so commonly encountered. The nuclei of the latter were never seen in the process of division.

Sections stained by van Gieson's method showed very little deposition of collagen fibrils in the central zone although dense concentric fibres were continually present at the periphery.

In lesions of longest standing fibrosis progresses and all that eventually remains of the pseudo-tubercle, is a dense concentric fibrous scar.

Between the individual tubercles fibrous granulation tissue is present infiltrated with eosinophils, fibroblasts, plasma cells, mononuclear cells, and occasional ova. Capillaries are numerous but do not seem to enter the pseudo-tubercles. It is common to find the whole appendicular wall involved in the more advanced cases in which pseudo-tubercles at all stages are seen, but the mucosa as a rule is not invaded by fibrous reaction. It may be pressed upon and the lumen distorted.

As a rule the mucosa in these cases is hyperplastic, and the nuclei of the epithelial cells are frequently seen in a state of mitotic division. The mucus cells are actively secreting, and the lymph nodes contain active germinal centres.

SUMMARY.

1. Appendicectomy was performed on thirty-five individuals inhabiting a hyperendemic area of bilharziasis in Northern Nigeria.

Ova of *S. haematobium* were present in the urines of all patients.

2. Twenty (57 per cent.) of the thirty-five appendices examined showed terminal spined ova.

3. Four (20 per cent.) of the twenty affected appendices had gross macroscopic lesions which were held accountable for the severe appendicular symptoms described.

Clinical and pathological details of these four cases are given.

4. Pathological technique and observations are recorded in detail. The histopathology is described and discussed.

5. A constructive account of the pathological process is given, and the stages illustrated by a series of photomicrographs.

CONCLUSIONS.

Bilharziasis causes its own type of appendicitis, the symptoms of which occasionally become urgent enough to warrant surgical intervention. The gross pathology encountered bears this out.

We are only in agreement with BARSOUM when he states that bilharziasis " does not cause or predispose to appendicitis " if this is taken to indicate purely pyogenic inflammations. Acute symptoms clinically indistinguishable from pyogenic appendicitis are in fact occasionally met with.

The lesions responsible in such instances result from ova infestation of the appendix upon which gross tissue changes have followed. Such gross bilharzial lesions of the appendix are infrequent : only when present do they cause appendicial symptoms that overshadow the commoner clinical manifestation of bilharzial dysentery.

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BERIBERI IN EGYPT.

BY

H. B. DAY, *M.C.*, M.D., F.R.C.P.,

Professor of Clinical Medicine, University of Cairo.

It is not uncommon in Egypt to encounter cases of general oedema that are not associated with cardiac or renal disease, but with certain endemic affections. Thus a general oedema may indicate the invasion period of bilharziasis, but it is usually so slight and transient that the patient rarely applies for hospital treatment. The associated features, some irregular fever and a high eosinophilia on blood examination, are sufficient to distinguish this variety.   More commonly a general oedema is found, with some cases of severe anaemia due to ankylostomiasis, partaking more of a nutritional character.   The serum protein is low, but the oedema appears to be largely due to anoxaemia from the extreme deficiency of haemoglobin.   Appropriate treatment with vermicides and iron brings about a rapid disappearance of the oedema, the excess fluid being discharged by the kidneys with consequent diuresis.

Lately several patients have been admitted to my wards with a severe and most extensive oedema accompanied by symptoms of beriberi.   There was no severe anaemia nor was the serum protein much reduced.   The oedema involved the whole body including the face and arms ; the heart was much dilated and there was some loss of the peripheral reflexes.   In each case the signs of pellagra were present and had preceded the appearance of oedema. The following cases are reported as examples :—

A male Soudanese, aged 23, resident in Cairo, had been incapacitated for 3 weeks with general weakness, shortness of breath and swelling of the face, body and limbs with some cough.   He was admitted to hospital on 8th February, 1936.

On examination there was considerable general oedema and ascites.   The patient was dyspnoeic, the veins in the neck were engorged and the liver enlarged and tender. The heart was very greatly dilated in both lateral directions with the apex in the mid-axillary line (see radiograph) and a pulse rate of 100, without definite murmurs.   The blood pressure was 120/90 ; an electro-cardiogram showed slight lengthening of the QRS complex with normal rhythm.

Blood examination : Haemoglobin 77 per cent. ; red corpuscles 6,000,000 ; white count 5,600, with 42 per cent. lymphocytes and 10 per cent. eosinophils.   The eosinophilia

was apparently due to bilharzial disease of the bladder ; there was no evidence of parasitic infestation of the intestine nor of renal disease.

There was no history of antecedent rheumatic manifestations nor of cardiac trouble ; the Wassermann reaction was negative. The skin over exposed areas showed the pigmentation and roughening characteristic of pellagra, and the tongue the smooth atrophic mucosa, while the nails were spoon-shaped. In pellagrins it is usual to find exaggeration of the tendon reflexes with a tendency to ankle clonus. But in this patient the knee jerks were barely obtained, while the ankle jerks were absent.

Treatment by digitalis was without effect on the cardiac condition. On 18th February marmite was begun and within a week the oedema, ascites and enlargement of the liver had disappeared. The great dilatation of the heart began to diminish and on 8th March, when the second radiograph was taken, the apex was only 1 finger breadth outside the nipple line. Convalescence was rapid and the patient put on flesh till his discharge.

Another patient, aged 20, admitted later, presented very similar symptoms. In this case the oedema was massive with hydrothorax, and the cardiac enlargement (which was extreme) appeared due as much to pericardial effusion as to dilatation. Marmite with digitalis produced no improvement and injections of liver extract failed to better the condition.

We were fortunate to secure a supply of Vitamin B_1 injections (ROCHE) and on this treatment, supplemented by salyrgan, a steady recovery ensued. The extensive dropsy disappeared with the loss of 50 lbs. in weight ; radiographs showed the great change in the cardiac outline as the pericardial effusion and ventricular dilatation subsided. Before his discharge a slight return of the ankle jerks was noted.

Other patients have shown similar symptoms but not of such severity that the diagnosis could be established with certainty. In these milder and doubtful cases the employment of the adrenalin test should be very useful, as practised by AALSMEER.

The association of beriberi with pellagra in these cases is of special interest and is not surprising in view of the defective diets common among the poorer classes in Egypt. This combination of two distinctive deficiency conditions has recently been described by CASTELLANOS of Cuba (1935) as " Pellagroid beriberi syndrome."* In the Egyptian cases the pellagra presented the usual symptoms with one exception, namely, the loss or great diminution of the tendon reflexes instead of the customary exaggeration. While the occurrence of a peripheral neuritis is described in the usual accounts of pellagra, it is quite an exceptional complication in Egypt. It is possible that this neuritis is dependent on a separate deficiency factor, seen in its greatest extent in the " dry " form of beriberi, but which may be present in slight degree in the dropsical variety of beriberi and in some cases of pellagra. Alcoholic neuritis has recently been attributed to the same cause.

The object of the present communication is to call attention to the existence of beriberi in Egypt, generally in combination with pellagra. The condition should be suspected when cardiac failure is associated with much general oedema and absence of signs of renal disease. The presence of pellagra, but with absent ankle jerks, was a distinctive feature in the cases described. Resistance to digitalis and recovery on Vitamin B treatment affords a therapeutic confirmation of the diagnosis.

* CASTELLANOS, A. *Bol. Soc. Cubana de Pediat.*, 7 Jan., 1935.

No. 1.

No. 2.

No. 1.—Radiograph taken 11th February, 1936.
No. 2.—Radiograph taken 8th March, 1936.
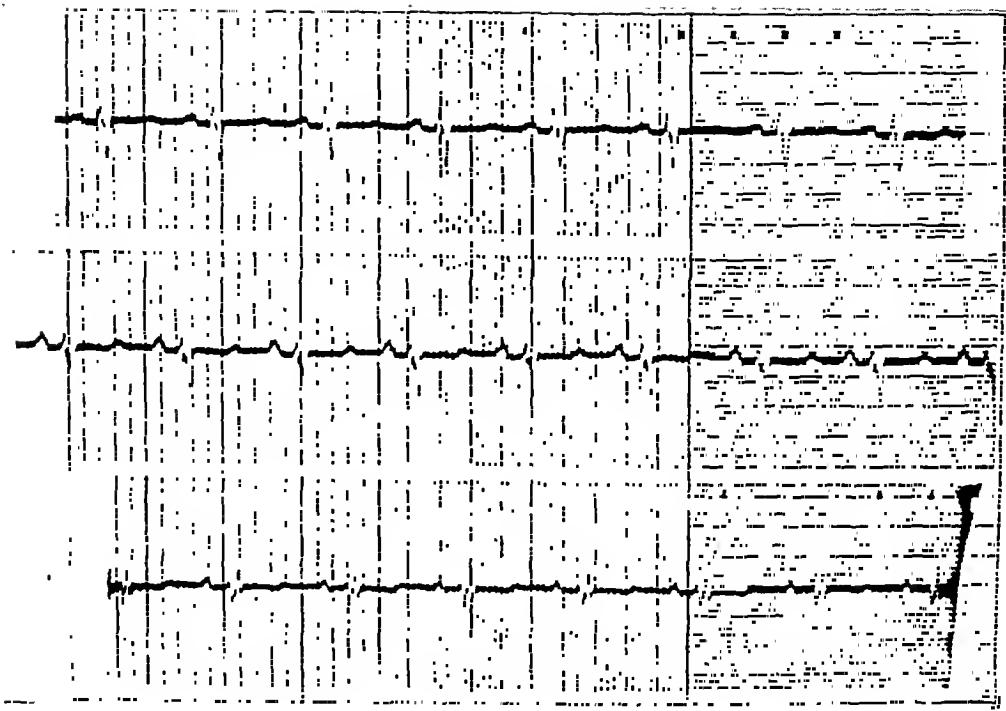
Electro-cardiogram (taken 13th February, 1936).

THE ANTIGENIC CHARACTERISTICS AND RELATIONSHIP OF VIPERINE VENOMS BASED ON THE CROSS NEUTRALIZING ACTION OF HETEROLOGOUS ANTIVENOMOUS SERA.

BY

E. GRASSET, M.D.,

AND

A. ZOUTENDYK,

From the Serum Department, South African Institute for Medical Research, Johannesburg.

In a paper recording the results of a comparative investigation on African and Indian venoms and antivenomous sera which has already appeared in these TRANSACTIONS (GRASSET and ZOUTENDYK, 1935) we intimated our intention of broadening the scope of this line of work so as to include cross neutralization experiments on venoms and sera from as many parts of the world as opportunity would allow.   This has now been made possible thanks to the friendly co-operation of Col. JOHN TAYLOR, Director of the Central Research Institute, Kasauli, India ; of Dr. OTTEN, Director of the Pasteur Institute, Bandoeng, Java; and of Dr. VITAL BRAZIL, Director of the Instituto Vital Brazil, Niteroi, Brazil.   We are also indebted to the Pasteur Institute, Paris, for supplies of various sera and venoms.   With the addition of our own local products we were thus in possession of a comprehensive selection of European, Asiatic, American and African venoms and antivenomous sera ; these included material of both viperine and colubrine origin, but these studies will be confined to the viperine venoms owing to their greater interest and complexity.   The

colubrine venoms possess properties in common which render the problem more simple and its practical importance of less moment. The Viperidae are so diverse and their venoms vary so much in ill-defined but fundamental characteristics that the group action exerted by antivenomous sera on heterologous viperine venoms is a subject of considerable immunological interest, and the therapeutic cross action is of extreme practical importance to those engaged in the production of polyvalent antivenomous sera.

In this study we were concerned only with homologous and heterologous neutralizing action in the hope that it might be possible to place Viperidae from different parts of the world into antigenically related groups and to draw some conclusions relating to the practical utility and limitations of the treatment of bites of snakes other than those against the venoms of which the sera are prepared. It was not practicable to consider other aspects of envenomation which have already been studied by numerous workers in many countries.

Details Relating to the Venoms and Antivenomous Sera Utilized for these Tests.

Europe.

Desiccated *Vipera aspis* venom (France).

Serum from the Pasteur Institute, Paris, marked " E.R." This serum is specially prepared for the treatment of bites inflicted by European vipers.

North Africa.

Desiccated venom of *Cerastes cornutus* (North Africa).

Serum from the Pasteur Institute, Paris, marked " A.N." designed for use in the treatment of bites inflicted by North African Viperidae.

South and Equatorial Africa.

Desiccated venoms of *Bitis arietans, Bitis gabonica* and *Causus rhombeatus.*

Polyvalent concentrated antivenene prepared at the South African Institute for Medical Research, Johannesburg. 1 c.c. of this serum neutralized either 10 mg. *Bitis arietans* venom or 20 mg. *Causus rhombeatus* venom.

Asia.

Desiccated venoms of *Vipera russelli* (India) and *Aucistrodon rhodostoma* (Java).

Polyvalent concentrated serum prepared at the Central Research Institute, Kasauli, India. 1 c.c. of this serum was stated to neutralize 4 mg. *V. russelli* venom.

Polyvalent serum prepared at the Pasteur Institute, Bandoeng, Java. 1 c.c. was stated to neutralize 0·1 mg. *A. rhodostoma* venom.

America.

Desiccated venoms of *Crotalus terrificus, Lachesis atrox* and *Lachesis jararaca* (Brazil).

Concentrated anti-bothrops serum prepared at the Instituto Vital Brazil, Brazil, 1 c.c. of the serum being claimed to neutralize 1·3 mg. *Bothrops* venom. Concentrated anti-crotalica serum also prepared at the Instituto Vital Brazil, 1 c.c. neutralizing 0·7 mg. crotaline venom.

TECHNIQUE ADOPTED FOR THE SPECIFIC AND CROSS NEUTRALIZING TESTS.

Every mixture of venom and serum was allowed to remain in contact for 1 hour at laboratory temperature. In the majority of cases rabbits weighing 2,000 grammes were injected in the marginal vein of the ear, but in all the tests employing South American venoms pigeons were injected intravenously.

The results afforded by the various tests have been grouped into three tables. Table I summarizes the average cross action exerted by seven different sera on European and African venoms ; Table II serves the same purpose in connection with the venoms of Asia and America, whilst in Table III an attempt is made to correlate the complete results in a comparative form.

Attention should be drawn to a number of factors which may influence the interpretations to be drawn from the three tables. Where a serum is described as being specific, the neutralizing value is usually that claimed by the producers of the serum, although in a very few instances no titre was stated, so that we have had to determine the neutralizing value according to our standard method. Where blank spaces occur in the tables we have preferred to use the limited amount of material at our disposal for the more detailed study of other cross neutralizing tests, since the blank spaces cover aspects of this study which have already been dealt with by other workers.

We have endeavoured to approach the limit of neutralization wherever possible but this has not been practicable in every case because of the limited amount of material available.

One factor which may influence the conclusions to be drawn from the tables is the variable nature and potency of the different sera employed in the tests, some being concentrated (globulin solution) and others natural. They were, moreover, standardized in different parts of the world by workers with different views on the test dose of venom to be employed, the temperature at which the mixtures should remain in contact, the time of contact and the type of animal or bird to be used for the tests (rabbits, mice, pigeons, etc.). However, although these points are of some theoretical importance they are of no practical significance since it was our object to compare the various sera as issued and, presumably, as they would be used in the treatment of snake bite (Table I, p. 351).

Discussion.

The results of this comparative study on the cross neutralizing excrted by various sera on viperine venoms from Europe, Africa, Asia and America, should be considered in the light of previous investigations undertaken by us and by other workers. Viewed as a whole, the results tend to show that although an antigenic similarity is frequently observed among Viperidae belonging to the same sub-family and genus, this is by no means the rule and antigenic relationship cannot be based on zoological grounds or on the geographical distribution of the reptiles. We have already shown (GRASSET and ZOUTENDYK, 1936) that the venoms of *Bitis arietans* and *Bitis gabonica*, which are classified zoologically in the same genus of the sub-family Viperinae, exhibit fundamental differences which are of great immunological significance. Similar observations have been made on European vipers (REUSS, 1930 ; OTTO, 1930, 1932 ; LAZAREVIĆ and BANIĆ, 1935) and on South American Viperidae (VITAL BRAZIL, 1902, 1907 ; KRAUS, 1930). On the other hand unexpectedly close antigenic relationships are sometimes observed where they would be contra-indicated by zoological classification and geographical distribution, examples which may be quoted being the European viper and South American *Bothrops* or the South American *Bothrops* and the North African *Cerastes*. These extremes of unexpected antigenic dissimilarity and equally unexpected antigenic similarity emphasize the importance of selecting viperine venoms strictly on their antigenic characteristics for purposes of serum production. It is, in our opinion, impossible in the present state of our knowledge to differentiate between " specificity " and " similarity." The obvious approach to this aspect of the problem is the biological investigation of the toxic and antigenic constituents of snake venoms leading to fractionation and identification of those substances which are specifically important in envenomation. Apart from the desirability of working with a known entity, the presence of aspecifie fractions merely serves to complicate the issue and to create unneeessary diffi-eulties. In our opinion, further progress can only be made by eoncentrating on those biological and biochemical aspeets to which an increasing number of workers interested in venoms and antivenomous serum therapeuties are now devoting their attention, and to which we hope to make a contribution in the near future.

TABLE I.

THE CROSS ACTION EXERTED BY ANTIVENOMOUS SERA FROM DIFFERENT SOURCES ON SNAKE VENOMS OF EUROPEAN AND AFRICAN ORIGIN.

Serum.	Venoms.		Equatorial and South Africa.		
	Europe.	North Africa.			
	V. aspis.	C. cornutus.	B. arietans.	B. gabonica.	C. rhombeatus.
Pasteur Institute, Paris, "A.N." serum	1 c.c. + 7 mg.	Specific 1 c.c. + 0·6 mg.	No action	No action	No action
Pasteur Institute, Paris, "E.R." serum	1 c.c. + 2 mg.	1 c.c. + 3 mg.	No action	2 c.c. + 5 mg.	No action
Research Institute, Kasauli, India	No action	1 c.c. + 1 mg.	4 c.c. + 2 mg.	1 c.c. + 5 mg.	4 c.c. + 20 mg.
South African Institute for Medical Research, Johannesburg	No action	No action	Specific 1 c.c. + 10 mg.	2 c.c. + 5 mg.	1 c.c. + 25 mg.
Instituto Vital Brazil, Anti-crotalica serum	1 c.c. + 1 mg.	No action	4 c.c. + 2 mg.	No action	No action
Instituto Vital Brazil, Anti-bothrops serum	1 c.c. + 1·3 mg.	1 c.c. + 2 mg.	5 c.c. + 2 mg.	No action	No action
Pasteur Institute, Bandoeng, Java	No action	No action	No action	1 c.c. + 5 mg.	No action

TABLE 11.

THE CROSS ACTION EXERTED BY ANTIVENOMOUS SERA FROM DIFFERENT SOURCES ON SNAKE VENOMS OF AMERICAN AND ASIATIC ORIGIN.

Serum.	Venoms.				
	Asia.		America.		
	V. russelli.	*A. rhodostoma.*	*C. terrificus.*	*L. atrox.*	*L. jararaca.*
Pasteur Institute, Paris, "A.N." serum	No action	1 c.c. + 0·07 mg.	1 c.c. + 0·01 mg.	1 c.c. + 2 mg.	1 c.c. + 0·1 mg.
Pasteur Institute, Paris, "E.R." serum	2 c.c. + 1 mg.	1 c.c. + 0·07 mg.	1 c.c. + 0·01 mg.	1 c.c. + 1 mg.	1 c.c. + 0·5 mg.
Research Institute, Kasauli, India	Specific 1 c.c. + 4 mg.	1 c.c. + 0·07 mg.	1 c.c. + 0·01 mg.	1 c.c. + 2 mg.	1 c.c. + 0·8 mg.
South African Institute for Medical Research, Johannesburg	No action	1 c.c. + 0·1 mg.	1 c.c. + 0·01 mg.	1 c.c. + 2 mg.	1 c.c. + 0·2 mg.
Instituto Vital Brazil, Anti-crotalica serum	No action	1 c.c. + 0·1 mg.	Specific 1 c.c. + 0·7 mg.		
Instituto Vital Brazil, Anti-bothrops serum	No action	1 c.c. + 0·07 mg.		Specific 1 c.c. stated to neutralize 1·3 mg. bothrops venom.	Specific
Pasteur Institute, Bandoeng, Java	No action	Specific 1 c.c. + 0·1 mg.	1 c.c. + 0·01 mg.	1 c.c. neutralizes more than 2 mg.	1 c.c. neutralizes more than 0·4 mg.

TABLE III.

THE CROSS ACTION EXERTED BY THE VARIOUS ANTIVENOMOUS SERA TABULATED IN A COMPARATIVE FORM.

Serum.	Europe.	North Africa.	Equatorial and South Africa.			Asia.		America.		
	V. aspis.	C. cornutus.	B. arietans.	B. gabonica.	C. rhombeatus.	V. russelli.	A. rhodostoma.	C. terrificus.	L. atrox.	L. jararaca.
Pasteur Institute, Paris, "A.N." serum		Specific ++++	O	O	O	O	+++	+	+++	+
Pasteur Institute, Paris, "E.R." serum	Specific ++++	+++	O	O+	O	++	+++	+	++	+++
Research Institute, Kasauli, India	+++	+++	O+	+	+	Specific ++++	+++	+	+++	++++
South African Institute for Medical Research, Johannesburg	O	+++	Specific ++++	O+	Specific ++++	O	++++	+	++	++
Instituto Vital Brazil, Anti-erotalica serum	++	O	O+	O	O	O	+++	Specific ++++		
Instituto Vital Brazil, Anti-bothrops serum	+++	++++	O+	O	O	O	+++		Specific ++++	Specific +++
Pasteur Institute, Bandoeng, Java	O	O	O	+	O	O	Specific ++++	+	+++++	+++++

Note:—O = no demonstrable neutralizing value.   O+ = feeble and ill defined cross-neutralization.   +, ++, +++, ++++ = increasing degrees of definite cross action, the last being approximately equivalent to a neutralization of specific therapeutic value.

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PATHS OF INFECTION OF THE CENTRAL NERVOUS SYSTEM IN YELLOW FEVER.

BY

G. M. FINDLAY

AND

A. F. MAHAFFY,*

Wellcome Bureau of Scientific Research, London.

During the past few years symptoms involving the central nervous system have occasionally been recorded in cases of yellow fever from West Africa by Stéfanopoulo and Mollaret (1935) and Findlay and Stern (1935).

Two possible explanations for the occurrence of these symptoms may be given ; either a strain of yellow fever virus with enhanced neurotropism is now present in West Africa, or the ordinary pantropic virus can, in certain circumstances, gain entrance to the central nervous system where it produces nervous lesions.   That such an involvement of the central nervous system can occur in rhesus monkeys has been shown experimentally by Findlay and Stern (1935) and independently by Penna (1936) who produced encephalitis in these animals by inoculating the pantropic virus intracerebrally after having first protected the viscera with yellow fever immune serum ; Stéfanopoulo and Mollaret (1935) also recorded spontaneous encephalitis in a monkey inoculated subcutaneously with pantropic virus.   In view of these findings and of the occurrence of meningo-encephalitis and other nervous sequelae in man, following injection of the neurotropic strain of yellow fever virus (Laigret, 1933, Mollaret and Findlay, 1936, L'Hermitte and Fribourg-Blanc, 1936, and Sorel, 1936), it is not without interest to examine ·more closely the

* Member of the staff of the International Health Division, Rockefeller Foundation, on temporary assignment to yellow fever investigations in London.

ways by which yellow fever virus may reach the central nervous system. Three possible routes of approach for viruses to the central nervous system are recognized: I. Centripetal spread of the virus along the peripheral nerves. II. Permeation of the haemato-encephalitic barrier. III. Spread from the naso-pharynx by the olfactory nerves.

I.—CENTRIPETAL SPREAD OF YELLOW FEVER VIRUS ALONG PERIPHERAL NERVES.

Evidence in the case of viruses such as those of rabies, poliomyelitis and herpes, indicates that there may be centripetal spread along nerves to the central nervous system and possibly centrifugal spread from the central nervous system to the periphery.

THEILER (1930), in his original observations on the distribution of yellow fever virus in mice, showed that after intracerebral inoculation virus was present at death only in the central nervous system, the adrenals and peripheral nerves *such as the sciatic. These observations have been amply confirmed in monkeys,* while, in addition, LLOYD and PENNA (1933) have found virus present at death in the salivary glands. All these sites are entirely consistent with a centrifugal spread along nerves from the central nervous system.

Evidence of centripetal spread of the yellow fever virus along spinal nerve fibres is, on the other hand, lacking. LLOYD and PENNA (1933) inoculated rhesus monkeys intrasciatically : the monkeys failed to develop encephalitis. Similar observations have been made in these laboratories. Two rhesus monkeys injected intrasciatically with 0·5 c.c. of a 20 per cent. suspension of mouse brain infected with neurotropic yellow fever virus showed no evidence of involvement of the central nervous system though virus was present for a few days in the peripheral blood stream and immune bodies to yellow fever subsequently developed in the blood. There is thus nothing to suggest that yellow fever virus can travel centripetally along spinal nerve fibres as do the viruses of rabies, poliomyelitis and herpes.

II.—PERMEATION OF THE HAEMATO-ENCEPHALITIC BARRIER.

Yellow fever virus can readily pass through the blood brain barrier when this is artificially ruptured by such gross traumata as are caused by the intracerebral inoculation of starch (SAWYER and LLOYD, 1931) or by diseases such as scurvy in the guineapig (FINDLAY, 1935). There is, of course, an enormous literature on variation in the permeability of the haemato-encephalitic barrier (*cf.* KATZENELBOGEN, 1935) and such conditions as extreme youth, exposure to coal gas, menstruation and anaphylactic shock have all been thought to increase the permeability of the barrier. It is of interest in this connection that THEILER (1930) found that in very young mice intraperitoneal inoculation of neurotropic yellow fever virus was followed by encephalitis ; the tendency for intracerebral

localization decreased with increasing age. FINDLAY (1935) also found that exposure of adult mice to coal gas increased the tendency to encephalitis after intraperitoneal inoculation of neurotropic virus.

In view of the results recorded in the next section, it is open to question whether such factors as youth and exposure to coal gas actually increase the permeability of the haemato-encephalitic barrier or whether they favour infection by the third route of approach to the central nervous system.

III.—SPREAD FROM THE NASO-PHARYNX BY THE OLFACTORY NERVES.

Neurotropic yellow fever virus, when instilled into the nostrils of monkeys and mice is capable of giving rise to encephalitis. Evidence obtained by FINDLAY and CLARKE (1935a) suggests that the spread occurs not by the actual axones but along the perineural lymph spaces of the olfactory nerves which, unlike the perineural lymphatics of the peripheral nerves, are thought to communicate directly with the cerebrospinal space (cf. ELMAN, 1923, ABEL, 1934, ABEL, et al, 1935). If yellow fever virus were excreted from the blood on to the olfactory mucosa there would thus be no reason why the virus should not pass up the neurolymph spaces of the olfactory nerves and infect the brain. Evidence that viruses may be excreted from the blood on to the nasal mucosa was obtained in the case of poliomyelitis by LENNETTE and HUDSON (1935) and in the case of equine encephalomyelitis by HURST (1936). FRANCIS and MAGILL (1935) also found that in human beings with Rift Valley fever virus may be present in the naso-pharynx ; since it is unlikely that Rift Valley fever actually multiplies in the naso-pharynx, it is probable that it passes from the blood on to the nasal mucosa. In the case of equine encephalomyelitis in guineapigs, HURST (1936) brought forward evidence to suggest that excretion on to the nasal mucosa and passage up the olfactory nerve fibres is the usual method of infection of the central nervous system.

In order to determine whether yellow fever virus can infect the central nervous system experiments were carried out in hedgehogs and in young mice from 3 to 4 weeks old.

In the hedgehog, FINDLAY and CLARKE (1935b) showed that intraperitoneal inoculation of neurotropic yellow fever virus is invariably followed by the development of encephalitis and death. Hedgehogs were, therefore, inoculated intraperitoneally with neurotropic virus and at intervals thereafter were killed. They were as far as possible exsanguinated and the brains removed. At the same time the nasal mucosa was scraped, the scrapings suspended in 1 in 10 serum-saline and the suspension, after light centrifugation, filtered through Berkefeld V filters. The blood, the filtrate from the nose, and the brain, were all tested for the presence of virus by intracerebral inoculation of mice, the brain, before inoculation, being divided into three parts representing fore-, mid- and hind-brain. The results are shown in Table I.

TABLE I.

DISTRIBUTION IN HEDGEHOGS OF NEUROTROPIC YELLOW FEVER VIRUS AFTER INTRAPERITONEAL INOCULATION.

Number of experiment.	Interval between inoculation and death in hours.	Distribution of virus.*				
		Nasal mucosa.	Blood.	Fore-brain.	Mid-brain.	Hind-brain.
1	24	1	4	4	1	2
2	48	0	4	4	3	3
3	96	0	2	3	1	3
4	144	0	0	4	4	4

* Four mice inoculated in each batch ; the number of mice dying in each batch is recorded.

It will be seen that in the hedgehog killed 24 hours after inoculation virus was obtained in small amount from the nasal mucosa, while the concentration of virus was at first rather greater in the fore-brain than in the mid- and hind-brain.

Somewhat similar experiments were carried out with baby mice though here there were difficulties in obtaining scrapings of nasal mucosa. Blood and brain were, therefore, tested for infectivity as in the case of hedgehogs.

The results are shown in Table II.

TABLE II.

DISTRIBUTION IN YOUNG MICE OF NEUROTROPIC YELLOW FEVER VIRUS AFTER INTRAPERITONEAL INOCULATION.

Number of experiment.	Interval between inoculation and death in hours.	Distribution of virus.*			
		Blood.	Fore-brain.	Mid-brain.	Hind-brain.
1	6	4	3	1	0
2	6	4	2	1	0
3	6	4	2	1	2
4	18	4	0	0	0
5	18	4	0	0	0
6	18	4	0	0	0
7	18	4	0	0	0
8	24	4	0	0	0
9	24	2	2	0	0
10	24	3	1	0	0
11	24	2	0	0	0
12	24	2	1	0	0
13	42	0	4	4	3
14	42	1	2	0	0
15	48	0	4	4	4
16	72	0	4	4	4
17	96	0	4	4	4

* Four mice inoculated in each batch ; the number of mice dying in each batch is recorded.

It will be seen that the time at which virus reaches the central nervous system is somewhat irregular but that 48 hours after inoculation virus is present in all parts of the brain though absent from the peripheral blood stream. When allowance has been made for the difficulty in removing the whole of the blood from the brain by the process merely of exsanguination, it will be seen that there is at first a greater tendency for virus to be present in the fore-brain than in the mid- or hind-brain. These results, therefore, parallel those previously obtained by FINDLAY and CLARKE (1935a) after intranasal instillation of neurotropic yellow fever virus.

More convincing results of the importance of the olfactory mucosa as a route of spread from the blood stream to the brain were obtained by employing a somewhat different experimental technique.

It has been found that by the nasal instillation of various chemicals such as picric acid, tannic acid, or sodium aluminium sulphate, it is possible to prevent infection in mice and monkeys when such viruses as equine encephalomyelitis and poliomyelitis are subsequently instilled into the nose (OLITSKY and COX, 1934, ARMSTRONG, 1935, ARMSTRONG and HARRISON, 1935 and 1936). If these observations are correct, it seemed not improbable that the nasal instillation of chemicals might also prevent viruses from passing up the olfactory nerves after being excreted on the nasal mucosa from the blood, and in fact, recent experiments by ARMSTRONG (1936) have shown that in monkeys the intranasal instillation of picric acid prevents intravenously inoculated poliomyelitis virus from reaching the central nervous system. In the case of yellow fever in mice, experiments were first carried out to see whether picric acid (0·32 per cent. in saline) instilled intranasally, was capable of preventing neurotropic yellow fever virus, also instilled intranasally, from reaching the central nervous system. In these experiments the mice were lightly anaesthetised with ether and the picric acid was instilled into the nostrils. Preliminary observations showed that when picric acid was intranasally instilled once only, 30 minutes before the nasal instillation of virus, very little protection was afforded against the development of encephalitic symptoms. If, however, picric acid is instilled at intervals of 24 hours for some days before the instillation of virus a considerable degree of protection is afforded (Table III). These findings are thus in agreement with those obtained with the viruses of equine encephalomyelitis and poliomyelitis.

Further experiments were then undertaken to determine whether intranasal instillation of picric acid in very young mice prevents the development of encephalitis, following intraperitoneal inoculation of neurotropic yellow fever virus. The baby mice were, therefore, treated intranasally with picric acid for 6 or 7 days prior to the intraperitoneal inoculation of neurotropic yellow fever virus.

From the result of the experiments shown in Table IV, it is seen that the nasal instillation of picric acid, does confer a certain protection against encephalitis following the intraperitoneal inoculation of neurotropic yellow fever virus.

TABLE III.

THE EFFECT OF INTRANASAL INSTILLATION OF PICRIC ACID (0·32 PER CENT.) ON THE SUBSEQUENT DEVELOPMENT OF ENCEPHALITIS IN ADULT MICE FOLLOWING INTRANASAL INSTILLATION OF NEUROTROPIC YELLOW FEVER VIRUS.

Number of experiment.	Mice treated with picric acid intranasally.			Control mice.	
	Number of instillations.	Number dying with encephalitis.	Number surviving.	Number dying with encephalitis.	Number surviving.
1	5	2	8	9	1
2	5	3	7	7	3
3	5	2	8	8	2
4	6	3	7	6	4
5	6	2	8	9	1
6	7	1	5	7	3
7	7	1	5	8	2
Total	... ...	14 (22·6 per cent.)	48 (77·4 per cent.)	55 (77·5 per cent.)	16 (22·5 per cent.)

TABLE IV.

THE ACTION OF PICRIC ACID (0·32 PER CENT.) IN PREVENTING ENCEPHALITIS IN VERY YOUNG MICE FOLLOWING THE INTRAPERITONEAL INOCULATION OF 0·2 C.C. OF A 20 PER CENT. SUSPENSION OF NEUROTROPIC YELLOW FEVER VIRUS.

Number of experiment.	Mice treated with picric acid intranasally.			Control mice.	
	Number of instillations.	Number dying with encephalitis.	Number surviving.	Number dying with encephalitis.	Number surviving.
1	7	1	6	10	0
2	6	2	8	9	1
3	7	6	6	11	1
4	6	2	4	6	0
5	6	6	6	12	2
Total	... ...	17 (36·0 per cent.)	30 (64·0 per cent.)	48 (92·5 per cent.)	4 (7·5 per cent.)

DISCUSSION.

The experiments here described suggest that, as in equine encephalomyelitis in guineapigs and poliomyelitis in monkeys, so also in yellow fever there may, in certain circumstances, be a passage of virus particles from the blood stream on to the nasal mucosa whence they are carried along the olfactory nerves to the brain.

The evidence for this indirect method of spread from the blood to the brain, derived as it is from experiments carried out along different lines, does not, of course, entirely exclude the passage of virus through the haemato-encephalitic barrier, a phenomenon that undoubtedly occurs when the barrier is damaged by injury or disease ; but, in its cumulative character, the evidence here brought forward certainly favours the view that excretion on to the nasal mucosa and passage thence by the olfactory nerves to the brain is a path of infection for the central nervous system of considerable importance even if it is not the only route by which yellow fever virus can reach the central nervous system.

CONCLUSIONS.

1. No evidence is obtainable that yellow fever virus travels centripetally along spinal nerves to the central nervous system.

2 Yellow fever may pass through the haemato-encephalitic barrier when this is damaged either by injury or disease.

3. It is suggested that yellow fever virus may escape from the blood stream on to the nasal mucosa and thence may pass by the olfactory nerves to the brain.

4. The experimental evidence in favour of this view is derived from the following findings :—

(a) The distribution of virus in the brains of young mice following intraperitoneal inoculation.

(b) The finding of virus after intraperitoneal inoculation on the nasal mucosa of the hedgehog.

(c) The fact that picric acid instilled intranasally prevents infection when yellow fever virus is subsequently instilled intranasally in adult mice.

(d) The fact that picric acid instilled intranasally also prevents infection when yellow fever virus is subsequently inoculated intraperitoneally in very young mice.

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AN EPIDEMIC OUTBREAK OF MURINE TYPHUS IN A LABOUR GROUP IN AN INLAND VILLAGE IN PALESTINE.

BY

I. J. KLIGLER

AND

R. COMAROFF,

From the Department of Hygiene and Bacteriology, Hebrew University, Jerusalem.

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The possibility that the rat may serve as a reservoir of typhus rickettsia was first suggested by MAXCY (1929), on the basis of his epidemiological studies of a typhus-like disease in south-eastern U.S.A. Subsequently, DYER (1931) and his associates discovered the virus in fleas collected from rats caught in a typhus focus. Shortly after this, MOOSER, CASTANEDA and ZINSSER (1931) reported the isolation of the virus from the brain of rats trapped in a prison in Mexico City, where cases of typhus occurred. It was thus shown that the form of typhus in Mexico and south-eastern U.S.A. was a disease of rats presumably transmitted to man by the rat flea. Another important point brought out by these studies was that this virus could be differentiated from the louse-borne virus by the peculiar scrotal lesions it produced in the guineapigs, first noted by NEILL (1917) and subsequently studied in detail by MOOSER (1928).

Following this work came the investigations of LÉPINE (1932) in Athens and of MARCANDIER and PIROT (1933) on board a Mediterranean man-of-war. LÉPINE showed that the sporadic cases of typhus occurring in Athens were associated with rats from which he recovered a virus producing the same scrotal reactions in guineapigs as the Mexican and American viruses. MARCANDIER and PIROT obtained a similar virus from rats caught on board the man-of-war where the sporadic cases of typhus occurred.

These investigations established the similarity or identity of New World and Mediterranean typhus. In both instances the disease is mild and sporadic in character. These sporadic cases are associated with rats harbouring a virus which differs in certain essentials from that obtained from louse-borne infections.

Because of the sporadic occurrence of these infections, the rat-borne disease has been termed *endemic* typhus in contra-distinction to the *epidemic*, louse-borne or Old World typhus. It is for this reason that special interest attaches to an outbreak of typhus in a village in the Jordan Valley which assumed the proportions of an epidemic but proved to be due to the rat-borne virus. This outbreak has a number of points of interest which warrant a more detailed description. It occurred about 60 miles inland and not along the coast. It had the proportions of an epidemic, but certain peculiarities differentiated it from louse-borne epidemics. Finally, it is the first time that the rat virus has been shown to exist in Palestine.

NATURE AND COURSE OF THE OUTBREAK.

The outbreak occurred in the village of Bethania situated in the Jordan Valley, south of the Sea of Galilee. It is a small agricultural settlement consisting of about six permanent families and a variable number of workers. The settlers live in individual mud-brick houses, while the workers are distributed in a number of wooden barracks and tents. The workers have a common kitchen and dining-room. At the time of the outbreak there was a group of fifty-eight workers consisting chiefly of recent immigrants ; they ranged between 18 and 30 years of age.

The first two cases occurred at the end of March, 1936 (22nd and 31st respectively). Between that time and 27th May, there occurred forty cases. The last case appeared almost a month later (24th June.) In all there were forty-three cases among a total of fifty-eight workers, or an incidence equal to 74 per cent.

The weekly number of cases was as follows :—

March 22nd to 31st	..	2	April 29th to May 5th	..	7
April 1st to 7th	..	3	May 6th to 12th	..	5
„ 8th to 14th	..	1	„ 13th to 19th	..	7
„ 15th to 21st	..	3	„ 20th to 26th	..	3
„ 22nd to 28th	..	5	„ 27th to June 2nd	..	6

The sex distribution was of interest. The group consisted of twenty-seven men and thirty-one women. The forty-three cases consisted of twenty-three men and twenty women. The incidence among men was, therefore, equivalent to 85 per cent. and among the women 64·5 per cent.

The peculiarities of the epidemic are apparent. It was distinctly a spring outbreak in contradistinction to the louse-borne infection which is essentially a winter disease. The epidemic curve is flat and irregular ; the epidemic required a whole month to get under way ; and terminated abruptly. The incidence among the men was higher than that among women. There were only two cases among the resident population, which numbered thirteen men, women and children. In other words : the epidemic was largely confined to the recently arrived labourers.

Although the people lived under wretched and miserable conditions, their bodies were quite clean. No lice were found on any of the patients. The obvious feature was the abundance of rats. The place was literally infested with them. There was a veritable rat epidemic. They were found everywhere—in the living quarters, adjacent storerooms, dining-room, etc.

Clinically the cases were mild. Some showed a typical exanthem, others were free. There were no toxic cases and no fatalities. All cases gave a positive Weil-Felix reaction.

Everything pointed, therefore, to the possibility that the outbreak was caused by the rats. Consequently, rats were caught and sent to the laboratory for examination.*

INFECTION IN RATS.

Two separate batches of rats were sent to the laboratory. The first, sent by railway, was delayed in arrival, and when it finally reached the laboratory only one rat was still alive. The brain and spleen of this rat were injected into guineapigs and rats with positive results. The guineapig developed a typical serotal swelling and fever. Smears and cultures from the tunica were positive for rickettsia. The protocol is as follows :—

On 31st May, 1936, the rat was killed with ether, the spleen and brain removed, triturated and emulsified in 10 c.c. sterile saline. One c.c. of the suspension was injected into a male guineapig and two rats respectively. On 10th June the temperature of the guineapig rose to 39·5°C. and the next day to 39·7° C. On 11th June one of the testicles was removed for culture and passage. Smears of the tunica showed many rickettsia ; on 19th June the tunica cultures were rich in rickettsia. The guineapig infected on 11th June developed a temperature on 16th June, after an incubation period of 5 days. There was marked serotal swelling. One testicle was removed under ether anaesthesia and smears of the tunica showed typical rickettsia. The course of the temperature of this guineapig from 16th June was as follows : 39·4° C. ; 39·8° C. ; 40·6° C. ; 40·6° C. ; 40·3° C. ; 39·4° C. ; 39·5° C. ; 38·5° C. ; 38·4° C. On 1st July it was reinfected with our laboratory European strain and proved immune. The passage strain is being carried on in the laboratory with typical scrotal swelling.

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* We are grateful to Mr. YOELI for much valuable help in catching and bringing the rats to the laboratory under difficult circumstances.

The second batch of rats was received on 4th June, 1936. Thirteen of these were alive. About fifteen fleas were removed. These rats were divided into four lots. The brains of each lot were mixed and injected into guineapigs and rats. All four lots gave positive infections ; in two of the lots positive cultures were obtained from the tunica of the guineapigs. The fleas were washed quickly in iodine and alcohol, triturated and injected into a guineapig and rats. Positive infection resulted in the rats. The protocols are given below :—

Lot 1 consisted of three rats. Brain and spleen were removed and emulsified in 10 c.c. saline. One male guineapig and two white rats were inoculated. On 16th and 17th June the guineapig showed a mild rise in temperature (39·5° C.), but no other reaction. Subsequently (1st July) it was infected with our European strain and proved immune.

On 18th June passage from one of the white rats was made to a guineapig and after 5 days it developed scrotal swelling ; the temperature rose to 40·1° C. and ran a typical course. It proved immune to a reinfection with a Mediterranean strain.

Lot 2 consisted of four rats. The procedure was the same. The guineapig died of a paratyphoid infection. On 16th June the white rats were killed and brain and spleen passed to a guineapig and two rats. On 21st June scrotal swelling appeared ; the temperature of the guineapig rose to 39·2° C. ; on 22nd June—39·5° C. ; and 23rd June—40° C. One of the testicles was removed for culture and passage. Tunica smears were positive for rickettsia ; on 7th July the cultures were also positive.

Lot 3 consisted of three rats. The procedure was the same. On 14th June the temperature rose to 39·1° C. ; 15th June—39·8° C. ; 16th June—39·6° C. ; slight scrotal swelling ; passage of testes and spleen to another male guineapig. On 22nd June the temperature was 39·3° C. ; 23rd June—39·5° C. ; 24th June—40·3° C. ; typical swelling. One testis removed for culture ; on 1st July culture positive. Infection ran normal course and the temperature dropped to normal (38·8° C.) on 29th June.

The brain and spleen of the white rats were also passed on 16th June to a guineapig and rats. The guineapig developed a typical infection—beginning 22nd June and ending 29th June. On 23rd June one testis was removed for tunica cultures and on 1st July the cultures were positive.

Lot 4 consisted of three rats.—Both the guineapig and rats died of peritonitis.

Of the three groups in which the animals did not succumb to secondary infections, all gave positive results In one case the guineapig developed a typical infection. In all cases the white rat proved more susceptible, and passage from the white rats to guineapigs produced typical infections with scrotal swelling.

INFECTION IN FLEAS.

Immediately after the rats were etherized they were searched for fleas. The fleas were removed to sterile tubes and washed with iodine, alcohol and then with sterile saline. They were then triturated and suspended in 3 c.c. saline. One c.c. of the suspension was injected into a guineapig and two white rats. The guineapig did not develop an infection. On subsequent reinfection (12th July) with our laboratory Mediterranean strain it proved immune (apparently it had passed through an inapparent infection).

The brain and spleen of the white rats were transferred on 18th June to a guineapig and rats. The guineapig developed a mild fever with slight swelling.

A testis was removed and passed to another guineapig. After 12 days a typical fever developed. On reinfection with our European strain these two guineapigs proved immune.

It is apparent from these results that there was an epidemic among the rats as well as among the men. Of the eleven rats examined at least four (most probably more) were infected. The fact that the fleas caused only a mild infection in the guineapig would suggest that the infection had run its course and that the virus was chiefly present in the brain. It is known that rickettsia may persist in the brain several weeks after the infection has run its course.

Discussion.

There is little doubt that the epidemic was caused by the rat virus. The mildness of the infection in man, the heavy infection in the rats, the presence of the virus in fleas—all point in one direction. The investigation started too late to isolate the virus from the human cases.

It is also clear that the virus belongs to the murine type. The extensive scrotal swelling produced in guineapigs and the infectivity of the virus for white rats differentiate it from the louse-borne virus.

Two points require clarification. Is the rat infection endemic or has it been introduced from the coast? There are indications that the disease has been endemic in the country all along and not merely confined to the coast. On the basis of the seasonal occurrence of typhus during the last 10 years we suggested (KLIGLER and OLITZKI, 1933) that an insect vector, other than the louse, was probably responsible for the sporadic cases of typhus. These cases appeared almost every year in the different parts of the country. It was most prevalent in 1926-27 in the northern section, and in 1928 in the southern section. Some eighteen cases occurred in the early autumn of 1926 in Nahalah, a village about 20 kilometres from Haifa. The figures for the 10 year period, by months, were as follows :—

Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
30	26	35	36	36	42	54	60	43	54	47	45

It is apparent that the months of highest prevalence are June to November, *i.e.*, the summer and autumn rather than the winter months of the year.

The second point is not so easily explained. It is difficult to account for so extensive an outbreak in the case of a disease not fatal to rats. The presumption is that the flea is the vector. But usually the flea leaves the rat only after it dies. Since, however, this infection is not fatal to rats, when do the fleas leave their hosts? This epidemic in man can only be accounted for by the simultaneous epidemic of rats who were themselves experiencing a rickettsia epidemic. The relatively large number of rats and the extensive infection shown to have existed among them led to this epidemic among the labourers who were in

constant contact with them.   At any rate it is important to note that under certain conditions epidemic outbreaks of murine typhus in man are possible.

SUMMARY.

An epidemic outbreak of typhus is described which proved to have been caused by the murine virus.   Although ordinarily this type of infection is sporadic, it appears that under peculiar conditions where there is a heavy infestation of rats suffering from an epidemic, the intimate contact between man and rat may lead to an epidemic of murine typhus in man.

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THE FOUNDATIONS OF ANTIMALARIAL WORK.

BY

C. STRICKLAND, M.A., M.D. (CANTAB.).

Professor of Medical Entomology, School of Tropical Medicine, Calcutta.

The insistent cry in Bengal for " flooding and flushing " in order to cope with the malaria menace has led to the development of this paper and in order to approach its theme one must, forthwith, be somewhat trite.

Wherever water flows over the land there is removal from it of such masses as can be carried away by the stream and wherever there is flowing water there is deposition of such material as the stream has no longer the power to maintain in suspension.   From the highest mountains to the seaface the two phenomena can be observed side by side, their separate effects of course being in conflict ;
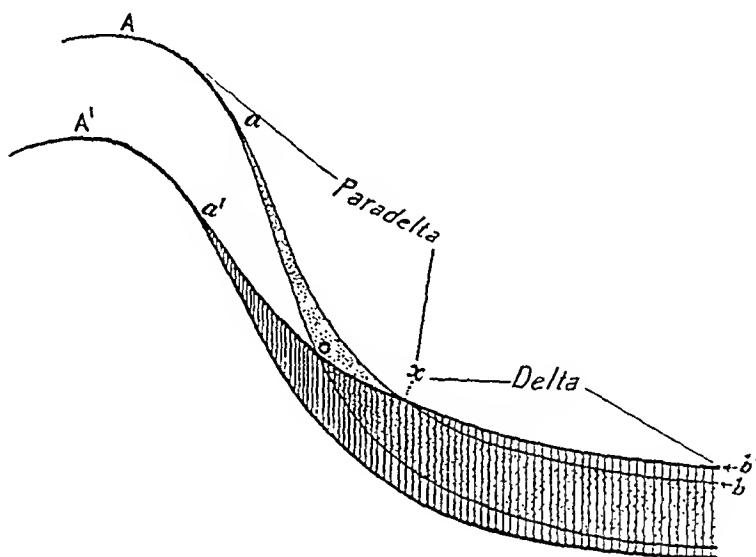
FIG. 1.—Diagrammatic representation of land profiles
at successive periods.

but one may say that " erosion " is transcendent over deposition in the hills, and deposition is the more powerful force in the plains, so that the general result in the hills is a lowering of the land relative to the sea, a loss of mass or " degradation," and in the plains elevation of the land relative to the sea, an accession of mass, or " accretion."

The deposits at any moment along a stream from its source in the highest mountains to the sea may be arbitrarily represented as in Fig. 1, in which the land

is shown at two successive stages, degradation on the one hand, in the hills, having taken place because of the transcendent erosion there ; and on the other, on the coastal plains, elevation or accretion, because of deposition prevailing over erosion. The region of such secular elevation (as in Fig. 1, b^1x) may be called the delta ; and the region A^1x, of secular degradation, the paradelta.

Now from a perusal of Fig. 1, it may be noted that the portion $a\ o\ x$ of the bed of alluvium which had at one time been deposited along the line of drainage was later on, during the process of degradation, washed away, and it may be a source of wonder that what had once been thrown down because of a lack of power of the transporting agent, the stream, should be subsequently removed.* Some reasons for the paradox are that the deposit of a moment becomes gradually comminuted and its particles rounded off and thereby rendered again transportable, partly that the stream attacks and grinds down the " bedrock " under the deposit which, *pari passu*, collapses, and partly that the deposit is sidetracked by the stream operating on its flanks (lateral planation) and cutting · down to a lower level thus undermining its previous plain (see Fig. 2). Therefore,
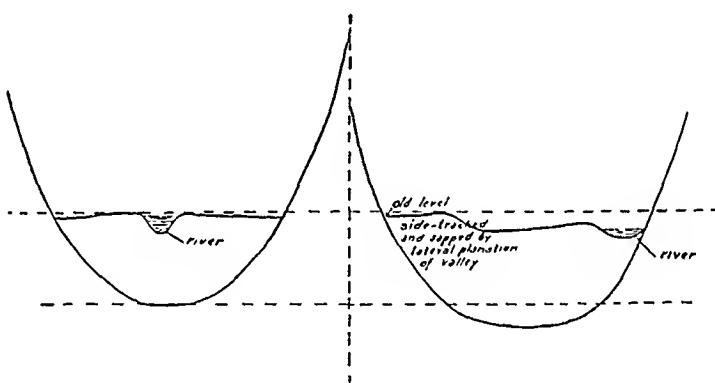
FIG. 2.—Diagrammatic representation of a valley at successive periods, showing degradation of recent alluvial deposits.

no alluvial deposit has any " divine right " to permanency, simply because it was once thrown down by a " wet nurse " that could bear the burden no longer. After all, the alluvial deposits of today are the sedimentary rocks of to-morrow, and there can be no doubt of the fact that these are subject to degradation.

It is sometimes an important point in relation to man's works (and this includes any designed for antimalarial purposes) to be able to tell whether the land is being raised or lowered concordantly with these considerations, and only this side of the subject is to be discussed here. The point is certainly not of

*Presuming, of course, that there had been no subsequent accession of power to the river or that no other factors had become operative.

PLATE I.

FIG. 1.—A *khal* in the Bengal delta showing strata of virgin soil, grasses and mangrove.

FIG. 2.—Showing *torá* with the gra *Oryza coacctata* in the background in vading the land, covered daily by the ti (River Hugli).

FIG. 3.—Showing a great landslide and fan, degradation and accretion both in the paradelta (the Teesta Valley, Sikkim).

PLATE II

FIG. 4.—Gnawing away of the land at the eye of a streamlet.

From Salisbury, 1907.

FIG. 5.—Avantipur, buried in the Vale of Kashmir.

much importance in a site where elevation or degradation is going on very slowly : if, for example, the land be undergoing degradation or accretion at the *net* rate of 1 foot *per millennium*,* one could well disregard the danger in this respect to any structure or engineering work. It is where accretion (as at the seaface) or degradation (as in the hills) is very active that the matter becomes of more practical importance. The general question, however, is to be discussed in this paper.

The problem would be simple enough if water having accession to the surface of the earth were to flow off it in a perfectly uniform mass. The paradelta would then be evenly washed away, the profiles everywhere would be as in Text Fig. 1, and the uniform loss could be ascertained at any point by merely having a vertical gauge embedded in the earth, while conversely the delta would be uniformly raised and the elevation could similarly be recognised by a gauge placed in the earth. As it is, however, the waters on the surface of the earth are differentiated into such entities as rivers, streams, and dewdrops, and into estuaries, *khals* and creeks, and as the greater the declivity and the volume of water carried in any channel the greater the amount of erosion in the paradelta and of deposition in the delta, so, along each line of drainage development proceeds at an independent rate, and deltaic and paradeltaic tracts at different stages of growth lie side by side like a jigsaw puzzle. For this reason evidence of what is happening at any one point along one line of drainage does not prove that the same changes are being enacted along neighbouring lines of drainage, and any conclusions one may come to at one point do not apply to a tract, and may, therefore, be of only limited practical application.

Keeping in mind this limitation to the value of conclusions one can draw from observations along a line of drainage, one may, however, consider what evidence bearing on the matter is available. Where the changes are rapid one may observe what is happening ; thus at the seaface it is obviously easy enough to tell whether the mud-banks are rising higher or not. They almost grow while one is looking at them, and this in spite of the fact that the level of the water is at the same time becoming ever higher by hydraulic action. Then animals and plants are sure indicators when the land is gradually rising, for every species has its proper range of habitat in relation to flooding. For instance, grasses fringe the young land as with a chaplet (Plate I, Fig. 1) and then, as it grows higher, one sees the *golpatta* palm (*Nipa fruticans*) ; *hargoza*, the sea-holly (*Acanthus ilicifolius*) ; and then the whole series of rhizophors (the mangroves) from *keora* (*Sonneratia apetala*) and *Torá* (*Kandelia redii*) that will thrive in water flooding its roots daily (Plate I, Fig. 2), *goran* (*Ceriops rogburghiana*) and *gengwa* (*Excoecaria agallocha*) up to *sundri* (*Heretiera litoralis*) that cannot thrive where there is more than occasional drenching of its roots

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*PIRSSON and SCHUCHERT (1920) say that the Ganges basin is being lowered at the rate of 1 foot *per* 1,750 years. This probably means an *average* degradation over its total area ; the actual degradation at its periphery would, of course, be at a much greater rate and at its bed at a much slower rate.

during the monsoon floods. On the other hand in the high hills one can infer the lowering of the land represented by great landslides from the mountainsides (Plate I, Fig. 3) or the gnawing away of the land at the eye of a streamlet (Plate II, Fig. 4).

But, except for such gross phenomena, the apparent evidences of secular accretion or degradation may be illusory, indeed, in the region where the secular changes are slow it is difficult to tell by any natural means whether the general
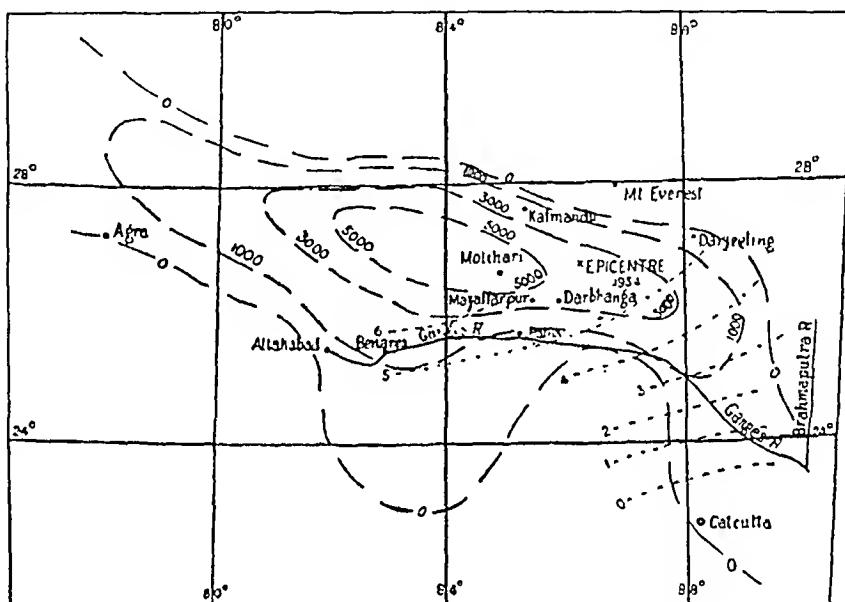
FIG. 3.—Sketch map showing main region of underload in Bengal, Bihar, etc., and secular changes of level in Bengal.
—— —— ——, contours of underload in feet of equivalent thickness of rock, density 2·67.
– – – – – – – – – –, generalised contours of secular change of crustal level in feet per century.

From De Graaff Hunter.

surface is being lowered or raised relative to the sea ; for example, the proximity of a strath, or *khadar*, or " flood-plain," of a river, to higher terraces, *bhangar*, or scarps, or hills, out of reach of the river-inundations that habitually flood its plain, does not prove that the plain has devolved from the higher terraces now left high and dry behind ; it might have arisen from lower levels by accretion and be now engaged in smothering the higher terraces* and hills.

Even the levels taken at points in relation to mean sea-level are not conclusive evidence, because the mean sea-level is not only subject to the changes that come

*With unconformable strata.

about during long ages* but, especially near the coast, to more rapidly acting influences such as the hydraulic rise following the contraction of the water-ways which proceeds with the elevation of the delta, or influences such as the hydraulic fall referred to by Sir SIDNEY BURRARD (1933) as due to the dredging of the Hugli estuary. If the base-level is thus inconstant, the relative levels of the land taken from time to time cannot be relied upon. Moreover, bench-marks on alluvium are subject to other errors. Deposits recently thrown down sink of their own weight and compressibility; for which reason, or for perhaps other reasons, subsidence has occurred in Bengal within the recent period, as proved by the fact that *sundri* trees (*Heritiera litoralis*) which are only viable a little above high tidal level are now found buried 16 feet below the surface.†
Even marks on " stable " rock formations cannot be certainly relied upon. The technical difficulties are irrefutably proved by the fact that while Dr. DE GRAAFF HUNTER (1934), Director of the Geodetic Survey of India considered as proved the raising of the levels in North Bengal and Bihar over a period of 70 years (see Text Fig. 3) Sir SIDNEY BURRARD (1934), late Surveyor General to the Government of India could not accept DE GRAAFF HUNTER'S conclusions.
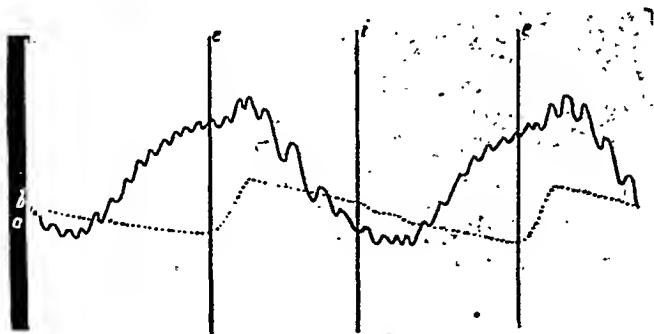
FIG. 4.—*a.* is the curve of blood pressure showing the long waves due to respiration and the short waves the heart beat.
b. is the curve of intra-thoracic pressure.
From M. Foster.

Ephemeral phenomena may mask the secular processes, just as the pulse-waves are minor features on the respiration curves of a blood-pressure graph (Text Fig. 4). For instance, every year there is a seasonal scouring out and silting up of the bed of the Hugli river.‡ Another example as illustrated in Plate I, Fig. 3 where an alluvial " fan " has arisen in a region of active degradation, to wit, the Teesta Valley of Sikkim.

*It is understood that the sea during the Ice Age, was about 100 metres below its present level.
†Similarly at the face of the Mississipi Delta stumps of cypress trees are found buried (TROWBRIDGE, 1930).
‡This incidentally is an example of how important these evanescent changes of the earth's surface may be to man, in this case to mariners.

With regard to such vicissitudes it may be remarked that as one proceeds towards the poles of the system they must be masked to an ever greater degree, on the one hand at the seaboard the accretion of the land is so rapid that any temporary accentuation of scouring tends to be nullified ; while in the high hills erosion is so rapid that temporary accretions to the land level at any point, for instance by landslide, can only be evanescent.

As yet another example, rivers of the delta may have their waters filched from them either naturally (by " capture ") or by man (by irrigation).   The floods then that had previously covered the land will recede to a lower level and expose new land, so that it may appear that the river has worn out for itself a deeper course and the land undergone degradation, although this will not have been the case ; and so the evidence of the oldest inhabitant in a locality on the occurrence of such changes may be credible as to facts but not valid for the point at issue.   He may have seen in his lifetime more and more land left behind by the river and becoming available for the culture of his luscious water-melons, but this would not necessarily have been due to the river having cut out for itself a deeper bed.   Conversely a river in the paradelta may become temporarily swollen and its plain heightened (as for example when it has captured another stream or when its basin has been deforested) and in this case the inundations of the land above the level of previous floods within the memory of man would not be sufficient evidence to allow the deduction that the general surface of the land was undergoing secular elevation.

One such ephemeral phenomenon may come about so rapidly that it is marked by the death of trees due to the blanketing of their roots with silt, as may be often seen in tin-mining areas where the tailings are carried over the land.

Further consideration of the matter shows that it is still further complicated by the tectonic processes of the earth's crust.

Either secular elevation or degradation of the land by water may be associated with either elevation or subsidence of the earth's crust by tectonism. The results of each possible combination is shown in the following table.

It will be seen then that changing levels relative to mean-sea-level do not always indicate either accretion or degradation by water-action, and it has been noted above that even if they do the technique of levelling may be fallacious.

" Sermons in stones," or the evidence afforded by old buildings or other masonry structures must now be considered.   If old cities or buildings were erected where secular elevation by deposition of sediment from water ensues they, of course, tend to become buried, whereas, if degradation occurs around the site, the foundations are eventually exposed and the buildings destroyed.   For example, if no other factors had been operative, secular elevation of the land would be proved by the burial of the temple at Avantipur (Plate II, Fig. 5) and of other temples in the Vale of Kashmir (the Jhelum Valley), or by the city of Patali-putra the capital of the Kingdom of Chandra Gupta over 2,000 years ago which was situated at the confluence of the River Soane and the Ganges near to modern

Patna, but was recently found buried below the general level of the ground. Such instances would appear to be conclusive evidence that the land has been elevated and that therefore the sites are in true deltaic tracts, but here again one must remember that the same result would have ensued if the elevation had been of only a temporary nature. Also, unfortunately, other influences may

TABLE.

Secular Change through Water Action.	Crustal Movement.	Change in Level of Water relative to Mean Sea Level.	Example.	Other Effects.	
A Accretion by net deposition	a Elevation	+	Carey Islands, Selangor, F.M.S.	Net deposition is sloweddown and becomes infinitesimally slow	
	b Subsidence	Depends on relative rapidity of A and b (i) It is —if A < b (ii) It is + if A > b	Sydney Harbour Mudbanks at seaface of Bengal delta		Same effect as B d
B Degradation by net erosion	c Elevation	Depends on relative rapidity of B and c (i) It is —if B > c (ii) It is +if B < c	Himalayan valleys ; the tower of Mt. Pelée (Fig. 10)		
	d Subsidence	—	Indo-Gangetic Trough	Net erosion is sloweddown and may become infinitesimally slow	Same effect as A b (i)

complicate such evidence. Wind-borne sediment may raise the general surface of the land and bury man's edifices as effectively as water-borne deposits without any relation of course to secular degradation or deposition by water.

Another possible fallacy regarding the evidence of burial of structures is that they may have wholly or partly sunk or crept under the surface of the earth

on which they were built. One can readily regard the deposits of earth, and especially those near a great river like the Ganges, as " quick," and that but a little more time is required for these " quick-muds " of the plains to swallow up denser bodies than would be the case if they were built on quick-sands along the sea-coast.

Moreover, buildings perish and fall down and plants grow upon their ruins and their humus gradually collects among them. This is added to by the dust and soil carried by the wind and eventually a mound marks the site where the building once stood. For instance, Taxila founded by Alexander the Macedonian, the ancient Buddhist *vihara* or monastery at Sanchi in Bhopal, and the site of Sarnath naar Benares, where Buddha first preached his message to mankind, have all become smothered by Aeolus and have needed excavation for the exposure of their structures.

It, therefore, must be in any case a matter of doubt whether a buried building or city or any masonry has been buried by the agency of water or otherwise. Thus an edifice, although built in the paradelta may be covered with wind-borne material and so perhaps may appear to be evidence of the elevation of the site by deltaic processes. The truth, however, might sometimes be arrived at from the fact that such deposits tend to cause mounds over structures. Thus WADDELL (1901) in his report on the excavation of Pataliputra, p. 11, said " Most of the leading landmarks of Asoka's palaces . . . remained so very obvious. . . ."

Wind, then, as an agent of transport of material may obscure the evidence afforded by buildings in the matter of hydraulic effects, but it is not only in this way that evidence may be obscured. The general surface of the land may be denuded here and elevated there by the agency of the wind. The effects may be here very slight or there gross, as for example, in the formation of sand-dunes. One must dissociate such elements from the problem, in studying the hydrographic changes.

Passing these matters in review then it will have been seen that, as the secular, or long-sustained, elevation or degradation of the land wrought by water may be of great immediate importance to man's livelihood—this being markedly the case at the poles of the hydrographic system of a country—the evidence which is available for recognizing in what direction Nature is working is of consequence to him. One sees, however, that except perhaps at the seaface and in the highest hills the evidence is equivocal. At the seaface there may be the indication afforded by the well-marked strata of plants and animals, but between the seaface and the high hills there is no good evidence. Thus the flood-plain of a river in a valley confined by hills is not necessarily evidence that the plain is in process of degradation, for it may be in process of elevation and engaged in smothering the foothills. Conclusions derived from levelling are prejudiced by changes in mean sea level and by the unreliability of bench-marks on compressible and subsiding alluvium (as is the case at least in the

Bengal and Mississipi deltas) and even by the unreliability, due to earthquakes of bench-marks on more stable rocks.

But quite apart from the difficulty of obtaining reliable evidence in the case it may be complicated by ephemeral and local hydrographic phenomena, such as floods due to deforestation and the capture of rivers.* The secular changes wrought by water may also be masked by crustal movements of the earth as, for instance, the subsidence of the great Indo-Gangetic trough, so much so that accretion by alluviation may sometimes proceed while the level relatively to the sea may be becoming lower, and conversely diluviation while the levels are rising, as for example, during the elevation of the spire of Mont Pelée (Fig 5). Of
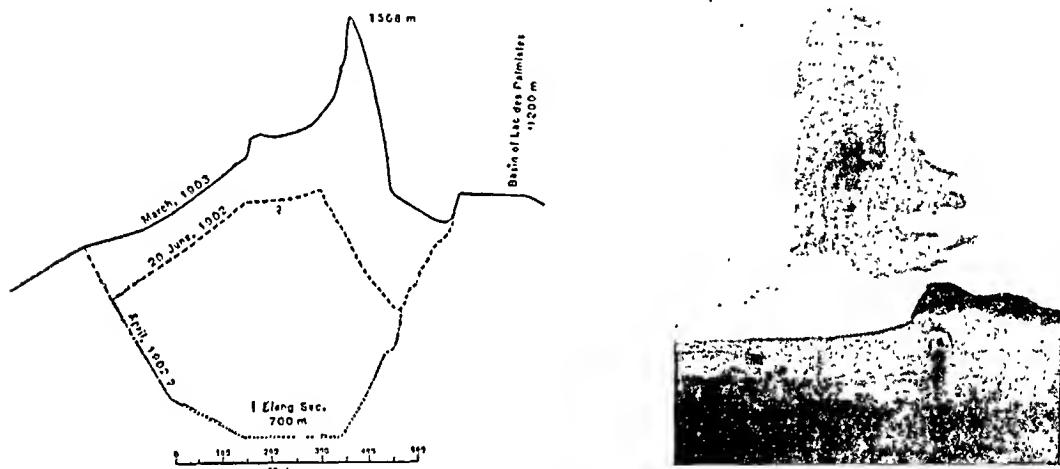
Cross-section through the northern part of Mt. Pelée, showing the growth of the spine.

Hovey, Am. Mus. Nat. Hist.

Spine of Mt. Pelée. The spine rose about 1,210 feet above the crater rim.

Hovey, Am. Mus. Nat. Hist.

Fig. 5.—THE TOWER OF MT. PELÉE, ELEVATION TRANSCENDING DEGRADATION.
From Salisbury, 1907.

the same order (in possibly masking evidence of the secular hydrographic changes) is the fact that wind may denude the surface of the land or cloak it with a deposit. Thus old cities, buildings or masonry structures that have become buried in the course of time might appear to indicate that there has been general accretion of the land by water if it were not that wind may have produced the same result. Nevertheless, buildings buried by aeolian action tend rather to have their sites marked by mounds than to become lost under a plain. It is necessary to state also that heavy masonry structures in the course of time sink bodily into the quick-sands and quick-muds of the earth's surface.

*Such phenomena also may, of course, be of great consequence to man and his works.

It is unfortunate then that while water action is the most important agent in the creation of the face of the land, the points of evidence that would seem to be available for indicating the antithetic tracts of accretion and degradation, the delta and the paradelta, are, except at the poles of the hydrographic system, equivocal.

Perhaps, after all, as man's works endure for but such a little while in the eternity during which Nature fashions the face of the earth, it is not in general of much moment to him to determine what courses she is pursuing. Nevertheless, it is important to understand the principles that guide her.

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SPLEEN INDEX AND OTHER MALARIAL PROBLEMS OF REMOTE VILLAGES ALONG THE ARAKAN COAST OF BURMA.*

BY

R. S. GREWAL, L.M.S. (Singapore), F.R.F.P.S. (Glas.),

District Medical Officer, Sandoway, Burma.

Introduction.

As no reports had been published on the malarial conditions prevailing in most of the villages along the Arakan coast of Burma owing to the great difficulty of reaching them, it was decided to visit the district with a view to studying the spleen rates and other relative problems affecting the population. Converting myself from a medical man into a mountaineer and *vice versa*, it has been possible, at considerable risk and trouble, to make a spleen census of many villages some of which had never before been visited by any medical man. No claim is made that the information gained by this study is by any means complete ; it is hoped, however, that the data collected may prove useful as a rough guide to malaria workers at some future date during any malaria survey or development of this part of Burma. It may be noted that there are twenty-one registered vendors of quinine in the district, but it appears that the sales of the drug are far from satisfactory.

Meteorological and Climatic Conditions.

In the absence of any meteorological station in the countryside reliance must be placed on figures obtainable at headquarters. These appear to be almost identical with those which would be obtained in the villages.

(*a*) *Rainfall.*—The rains commence in May and last till the middle of November. The total rainfall during a year varies from 170 to 250 inches. It was 244·06 inches during last year.

(*b*) *Temperature.*—The maximum temperature is never above 100° F., while the minimum temperature varies from 40° to 50° F. The summer season begins about the middle of March and lasts till the advent of the rains. There is a short winter season from the middle of December till the middle of March.

(*c*) *Humidity.*—It is cent. per cent. during the rains and in winter. In the dry season it is between 60 and 70 per cent.

Geographical Conditions.

The country is very hilly and covered with thick jungle. Communications are risky and difficult. Villages are situated on the sandy sea shore and at the foot of the hills where the people are able to cultivate paddy fields, their main occupation during the rains.

The following eleven villages were visited :—

(a) Gwa, (b) Taungup, (c) Natmaw, (d) Kyaukchun, (e) Pauktu, Dotan and Kyaukshut, (f) Yahaingkado and Yahaingkwin, (g) Taungbauk, (h) Aleywa, (i) Kanseik and Ashebet, (j) Kwingyi and Padegaw, (k) Sandoway Town.

Method of Investigation.

It was at first decided to adopt the well-known method for estimating spleen enlargement advocated by the celebrated DEMPSTER and used by him in the United Provinces in 1848 and characterized by CHRISTOPHERS as a " Unique method of measurement of disease." It consists in measuring the distance of the apex of the spleen from the umbilicus. This procedure had, however, to be abandoned in the very early part of the investigation owing to the difficulty of persuading the children to submit to the necessary examination. They appeared to be awe-stricken at the idea of this ordeal. Reliance had, therefore, to be placed on DARLING's finger breadth method. The children, consisting of males and females from the age of 1 to 10 years, were collected with the aid of the Headmen or " Thugyies." The group included children from the schools or " kyaungs " and "phongyi kyaungs," i.e., schools attached to the monasteries. No house-to-house inspection was made.

Spleen Index Figures.

Of 1,541 children examined in the villages, 178 were found to have enlarged spleens, thus giving an index of 11·55.

TABLE A.

Name of village.	Number of children examined.	Number of enlarged spleens.	Percentage.
Gwa	363	14	3·8
Taungup	448	25	5·58
Natmaw	83	7	8·43
Kyaukchun	25	8	32·0
Pauktu, Dotan and Kyaukshut	21	8	38·95
Yahaingkado and Yahaingkwin	61	19	31·14
Taungbauk	17	5	29·41
Ahleywa	15	3	20·0
Kanseik and Ashebet	47	2	4·25
Kwingyi and Padegaw	28	24	85·71
Sandoway Town	434	63	14·5
Total	1,541	178	11·55

From Table A it will be noticed that the Kwingyi and Padegaw villages which are situated at the foot of the hills are highly malarious and give the highest index. They were responsible even for inoculating the writer with malaria despite very strict precautions. Kyaukshut and Dotan and Pauktu are also highly malarious (38·95 and 32·0).

It is interesting to note in this connection that UTTLEY (1935) writing of a spleen survey in the colony of Hongkong noted that the villages situated at the foot of the hills were more malarious than others.

FEEGRADE (1927) in his report on malaria in Kyaukpyu Town, noted that children between 7 and 10 years of age gave a high spleen rate. He interpreted this as due to new infections. The high spleen rate in children between 6 and 7 years of age in my series (Table C, 14·96) can be accounted for in a similar manner. It will be noted from Table B that the degree of enlargement of the spleen was two finger breadths in the majority of my cases.

TABLE B.

SHOWING SIZE OF ENLARGED SPLEENS.

TOTAL CHILDREN EXAMINED 1,541.

Finger breadths.	Number.	Percentage.
1	48	3·1
2	81	5·27
3	32	2·08
4	12	0·78
Umbilicus	2	0·13
Below umbilicus	3	0·18

Parasite Index.—As difficulties were encountered in making the spleen examination it is not surprising that it was almost impossible to make blood films. Nevertheless nine were secured from the various villages by methods of subterfuge familiar to most medical men who are accustomed to blood-film work. These specimens were stained and examined in detail with the following results :—

Benign tertian, 5 ; benign and malignant tertian, 1 ; negative 3. Total, 9.

TABLE C.

Ages.	Children examined.	Number of enlarged spleens.	Percentage.
0–1	76	5	6·57
1–2	130	11	8·46
2–3	142	21	14·78
3–4	175	20	11·42
4–5	222	18	8·1
5–6	175	25	14·28
6–7	147	22	14·96
7–8	134	18	13·43
8–9	114	15	13·15
9–10	126	14	11·11
Over 10	100	9	9
	1,541	178	11·55

MOSQUITOES AND BREEDING PLACES.

In the absence of any special equipment and aid it was impracticable to make collections of mosquitoes in the villages, but a single specimen of a mosquito, which was found to be *Anopheles hyrcanus* var. *nigerrimus,* was taken in Sandoway Town. This mosquito has not been found infected in nature in Burma. Of mosquito breeding places, apart from the usual drains, pools and tanks, two are of great importance in Burma. The first is the uncultivated paddy fields to which attention was first drawn by JOLLY (1927) in his report on the malaria problem in Kyaukpyu, the adjoining district ; my own observations agree with his contention. A breeding place of still greater importance, however, is the " cup-shaped stump " which is left when the *dhani* tree is cut. This is one of the most favourite breeding places. It is very often overlooked and is one to which more attention should be paid by future workers. The *dhani* tree is very common in Burma specially in low lying and swampy places. It is a tree akin to cocoanut, its branches being used for roofing huts.

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THE RELATION OF BLOOD-CALCIUM TO TROPICAL ULCER.

BY

M. ELLIS,*

Colonial Medical Service, West Africa Section, Bida, Northern Provinces, Nigeria.

I.—ORIGIN OF RESEARCH.

The investigation to be described in this paper had its origin in three statements which had been made regarding calcium deficiency. The first was in the article on tropical ulcer in Price's Medicine (1933) which stated that many cases of tropical ulcer had a low blood-calcium ; the second was in the report by McCulloch (1933) on the food-stuffs of Nigeria which pointed out that most of the vegetable foodstuffs of Nigeria, with one or two outstanding exceptions were deficient in calcium ; the third was in a private communication from Dr. A. C. Lovett-Campbell of this Service, who informed me that he was obtaining good results in the treatment of tropical

ulcers by the intravenous injection of calcium. If, therefore, food-stuffs in Nigeria are deficient in calcium, it would seem reasonable that this deficiency would produce a low blood-calcium and the injection of calcium would be a rational line of treatment. In this investigation, an attempt to correlate the level of blood-calcium and the rate of healing of tropical ulcers has been made.

II.—METHODS EMPLOYED.

A standard line of treatment of the ulcer was adopted throughout. The basic principles are taken from the paper by CONNELL and BUCHANAN (1933), to whom I have pleasure in acknowledging my indebtedness. As most of the cases treated were out-patients with ulcers situated around the ankle, it was felt that to encase the ulcer in plaster of Paris and permit them to go away for some length of time was not wise. The following routine was therefore adopted At the first attendance, fomentations were applied to the ulcer to clear away earth or other native remedies. The next day, all the applications having been removed, the ulcer was measured, its area and perimeter being determined on the lines laid down by CONNELL and BUCHANAN. A zipp dressing and a firm bandage were then applied. The patient was instructed to attend at weekly intervals for renewal of dressings and inspection, until the ulcer was healed. A specimen of blood for blood-calcium investigation was taken on the first or second day.

III.—TYPE OF PATIENT TREATED.

The work was carried out at the Bida Native Administration Hospital in the Niger Province of Nigeria. The patients were largely Nupes, but there were a few Hausas, Yorubas, Kanuri and Pagans. They varied in age from 6 years to 50 years.

The sexes were about equally represented.

IV.—TYPE OF ULCER TREATED.

The ulcers treated were those seen in the out-patient department. No special selection was made except for the exclusion of those which were too small to be considered of value. In addition, however, to forty-one cases which presented themselves as out-patients for treatment for their ulcers, there were a further nine cases, the findings in which are set down in Table III. These consisted of cases from the Bida Elementary School and Middle School, who originally presented themselves for some trivial wound or abrasions. They were treated with flavine dressings according to the routine with such cases. In these nine cases, it was found on removal of the dressing after 3 or 4 days that the trivial wound or abrasion was spreading and progressing towards the stage when it could be dignified by the name of an ulcer. When this stage had been reached, a specimen of blood was taken immediately for an estimation of blood-

calcium, in order to determine whether there was any correlation between the level of the blood-calcium and the transformation of the wounds into ulcers. As these " ulcers " were small and quickly healed with a zipp dressing, they were not considered to be of value for observations on their rate of healing.

V.—PATHOLOGICAL CONSIDERATIONS.

Unfortunately, the Bida Hospital is situated about 250 miles from the nearest fully equipped pathological laboratory. It was not, therefore, possible to carry out routine Kahn investigations on these patients. Nor, as the author was working single-handed, was it possible to carry out full haematological observations. One or two cases showed obvious signs of syphilis and were treated accordingly. Bida is not a yaws area and, therefore, the effect of yaws in the pathogenesis and healing of the ulcers can be largely discounted. Most cases were treated for bilharziasis or ankylostomiasis if these conditions were found.

The blood-calcium investigations were carried out by Kramer Tisdall technique. An approximately $\frac{N}{10}$ solution of potassium permanganate was made up fresh at intervals of about 1 month. Each day, before using, this solution was carefully standardized against a standard $\frac{N}{10}$ solution of oxalic acid and then diluted with distilled water to a strength of $\frac{N}{100}$ before titration.

VI.—DISCUSSION OF RESULTS.

General.—The results obtained are set out in three Tables. The cases, the results from which are recorded in Table III, have already been described. Of the remaining forty-one cases out of the fifty taken for observation, only twenty-three remained under treatment and observation until the ulcer was finally healed. The results of these cases are recorded in Table I. The " healing coefficient " is worked out as described by CONNELL and BUCHANAN (1933) and forms a serviceable criterion of the rate of healing of the ulcers in inches per day. (This healing coefficient is multiplied by 100 in the Table to avoid an undue number of decimal places.) In the Table, the cases have been arranged in descending order of magnitude of the healing coefficient for easy comparison.

In Table II, the remaining eighteen cases who disappeared during treatment are set out with a bald statement of the blood-calcium level found and any other facts of importance.

CANTAROW (1933) in his book on calcium metabolism gives the normal for blood-serum-calcium as lying between 9 and 11 mg. per 100 c.c. These figures are determined from the examination of sixty-eight healthy Americans. In the absence of observations to the contrary, there is no reason to suppose

TABLE I.

Case number.	Days to heal.	Area in square inches.	Perimeter in inches.	Healing coefficient × 100.	Serum-calcium mg. per 100 c.c.
1	30	3·3	7·4	1·44	10·5
3	58	5·88	9·8	1·34	11·2
8	8	0·16	1·6	1·25	9·0
23	30	2·21	6·0	1·23	8·2
13	14	0·45	2·6	1·17	8·85
11	58	6·9	10·6	1·11	10·9
19	69	8·75	12·0	1·05	9·6
2	14	0·25	2·0	0·9	9·5
12	95	11·47	13·6	0·89	8·9
23	23	0·64	3·2	0·87	9·3
5	63	4·3	8·2	0·86	10·9
8	63	4·6	8·6	0·85	9·0
18	20	0·4	2·6	0·77	9·0
20	47	2·0	6·0	0·71	11·0
21	32	2·86	7·0	0·71	9·7
6	30	0·72	3·6	0·66	11·0
22	100	6·25	10·0	0·63	9·4
15	39	1·05	4·4	0·61	9·8
16	30	2·04	5·8	0·60	12·7
9	96	5·0	9·0	0·56	8·85
10	53	1·3	4·6	0·53	10·0
4	35	0·42	2·6	0·46	10·0
17	90	3·0	7·4	0·45	9·2

TABLE II.

Case number.	Blood-calcium.	Remarks.	Case number.	Blood-calcium.	Remarks.
45	8·7		32	11·5	
24	10·1	7 months' history.	33	10·3	
25	10·0	1 year's history.	34	8·2	
26	8·2	6 years' history : much scarring.	35	9·2	Obvious signs of syphilis.
			36	9·6	
27	10·1		37	10·4	Obvious signs of syphilis.
28	9·2	Slow to heal.	38	8·9	
29	9·8	Ulcer spread rapidly.	39	7·5	
30	9·2	15 years' history.	42	8·8	
31	8·0				

TABLE III.

Case number.	Blood-calcium.	Case number.	Blood-calcium.
40	9·9	47	14·0
41	9·9	48	8·9
43	7·8	49	9·0
44	11·2	50	9·0
46	8·5		

that the findings in Africans will be radically different from these. Considering therefore, Tables I, II and III together, it will be seen that of fifty cases of ulcer, thirty-one cases give figures which lie between these limits, five cases give figures which are above these limits, and thirteen cases figures below these limits. These figures, therefore, suggest in this series of cases that the level of blood-serum-calcium has no part in the pathogenesis or establishment of the ulcer. The figures in Table III are of particular interest for here we can observe ulcers in the making. Out of these nine cases, four cases had a serum-calcium within normal limits ; two cases were above and three cases were below these limits. It would seem definite in these cases that a deficiency or excess of calcium played no part in establishing the ulcer.

Considering the figures in Table I, the general impression gained by a study of the table is that the level of the serum-calcium is in no way related to the healing of the ulcer. Comparing the first six cases in the table with the last six, it will be seen that the rate of healing of one group is approximately twice that of the second. And yet in the first six cases, there are three in which the serum-calcium is normal, two in which it is low and one in which it is high. In the last six, there are four cases with a normal serum-calcium, and one each of high and low calcium.

In Table II, we find that Cases 24, 25, 26 and 30 are of long duration. Three of them have a normal serum-calcium and one has a low serum-calcium. Cases 28 and 29 have normal serum-calciums, but one was observed to be slow to heal and one was spreading rapidly under treatment.

It is concluded, therefore, that the level of the serum-calcium is of no value in determining the rate of healing of these ulcers. If that is so, then the intravenous injection of calcium salts is not a rational line of treatment for tropical ulcers.

VII.—SUMMARY.

1. Fifty cases of tropical ulcer in being or in formation were selected for estimation of the blood-serum-calcium. The values found showed that the level of the serum-calcium is not a factor in the pathogenesis.

2. Twenty-three out of the fifty cases were observed until they healed. The rates of healing were found to bear no relation to the level of serum-calcium.

3. Eighteen cases observed for only part of the time occupied in healing confirmed the statement made in paragraph 2.

4. It is concluded, therefore, that intravenous injection of calcium salts in the treatment of tropical ulcer has no rational basis.

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CORRESPONDENCE.

ZINSSER'S INTERPRETATION OF THE ANGLO-SAXON WORD 'DRIF.'

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

In his book, *Rats, Lice, and History*, Professor HANS ZINSSER quotes at some length from a paper by me which appeared in the TRANSACTIONS (1927, Vol. XX, p. 487), under the title, "*Old-time Typhus in Britain.*" In this paper I referred to the famine-pestilence briefly recorded in *The Anglo-Saxon Chronicle* for the year 1087, which in the original text is called *drif*. This word I translated by "fever," and suggested, therefore, that the outbreak was probably of the same nature as later famine-fevers which we know beyond question to have been typhus. Professor ZINSSER, in the most courteous manner, disagrees with me here, and states that *drif* meant "diarrhoea." He argues accordingly that "this [epidemic] was quite evidently not typhus." No authority is given for his interpretation, but I suspect that it originates ultimately from Ingram's English version of this chronicle (published over a century ago), where *drif* is translated "diarrhoea," wrongly, as I maintain.

Since Professor ZINSSER's learned and fascinating treatise has been read widely by members of our Society, I feel that I should make it clear to these that I was not in error. I could call to witness on my behalf, BOSWORTH's and CLARK HALL's authoritative Anglo-Saxon dictionaries, as well as THORPE's official translation of the *Chronicle* published with the imprimatur of the British Museum, in all of which *drif* is translated "fever." But in a matter of this kind it is preferable, when possible, not to rely on the opinion of modern scholars, however numerous and learned. Infinitely more weighty would be the silent testimony of one who, skilled in Latin, and with Anglo-Saxon as his mother tongue, has left some manuscript in which textual matter containing the word in dispute, appears in both languages. Such conclusive evidence regarding the point at issue is to be found in the text known as the Rushworth Codex. This is an ancient Latin MS. of the Gospels in which some early English cleric interlineated a word-for-word translation into Anglo-Saxon. The original Latin is written in a large, bold hand, and above each word there is added in tiny characters the Anglo-Saxon equivalent. Here in more than one instance, *febris* is translated by *drif*, but a single example will suffice. The first sentence of Matt. viii, 15, runs :—

> And æthrán honda his and forlet hiae sio drif.
> *Et tetigit manum ejus et dimisit eam febris.*

Lest anyone should imagine that the prefaced " *sio* " might materially affect the signification of *drif*, I would point out that it is merely the feminine form of " the."

I submit that this evidence puts the meaning of the word in question beyond all doubt.

I am, etc.,

Royal Army Medical College,   W. P. Mac Arthur.
*Millbank, S.W.*1.
19*th October,* 1936.

REVIEW.

PLAGUE.

A MANUAL FOR MEDICAL AND PUBLIC HEALTH WORKERS.*

It would be impossible to give an adequate review of this large treatise, nor indeed is any lengthy account of the work necessary. The name of its chief begetter, Dr. Wu Lien-Teh, is in itself a sufficient guarantee of merit. Every aspect of plague is covered in comprehensive fashion—Bacteriology, Immunology, Pathology, Animal hosts and vectors, Epidemiology, Prophylaxis, Management of epidemics, with, of course, a full clinical account. The many illustrations are clear and to the point, and to each section there is appended an extensive list of bibliographical references.

The authors are to be congratulated on the production of a volume which will prove a trusty staff to those who, in whatever capacity, are concerned with plague.

W. P. M.

Proceedings of a Clinical and Laboratory Meeting of the Society,
held at the Hospital for Tropical Diseases, Gordon Street, London, W.C.,
on Thursday, 19th November, 1936.
Sir Arthur Bagshawe, *C.M.G.*, M.B., D.P.H., *President*,
in the Chair.

DEMONSTRATIONS.

Dr. H. A. Baylis.
Microfilariae in the liver of a chamaeleon.

A section was shown of the liver of a chamaeleon from Tanganyika Territory, in which microfilariae were present in the blood. The parent worm was not obtained, and the specific identity of the microfilaria is therefore unknown.

Major H. C. Brown.
A simple method for the determination of the sign of the electric charge of trypanosomes in the blood of an infected animal.

This is a slight modification of the method described by J. C. Broom, H. C. Brown and C. A. Hoare (1936) in these Transactions, xxx, 87.

Put one drop of blood containing trypanosomes into 0·5 c.c. of glucose saline (one volume normal saline, nine volumes 4 per cent. glucose in distilled water) : allow to stand for 3 minutes. Examine under coverslip with 1/6 inch objective. Negatively charged trypanosomes are free from the red cells. Positively charged trypanosomes are firmly adherent to the red cells.

Dr. C. C. Chesterman.

Masses of rubber found in stomach and intestine of natives in the Belgian Congo following introduction of latex. (See Communication on the subject on p. 475.)

Drs. W. E. Cooke, A. L. Gregg and P. Manson-Bahr.

Three cases of ambulant trypanosomiasis, *T. gambiense.*

These cases form the subject of the Communication on p. 461.

Dr. G. R. Mather Cordiner.

X-rays of gastro-intestinal lesions.

1. Mucosal relief of normal large intestine.
2. Mucosal relief in a case of ulcerative colitis.
3. Mucosal relief of large intestine showing pseudo-polyposis.
4. Opaque enema with colon filled, showing filling defect in transverse colon and diverticula of descending colon.
5. Same case, combined barium and air distention showing filling defect to be due to an adenomatous polyp.
6. Radiogram of dilated coils of small intestine—a result of obstruction of ileo-caecal junction.

Prof. L. J. Davis.

Microscopic preparations were shown illustrating the following conditions as seen in certain laboratory animals in Hongkong.

(*a*) *Clonorchis sinensis* infection of the livers of cats.

(*b*) Lung mites of the genus *Pneumonyssus* in Macaque monkeys.

(*c*) *Klossiella muris* in the kidneys of mice.

(*d*) *Bartonella*-like bodies in the red corpuscles of a guineapig. They had become more numerous following splenectomy.

Dr. N. Hamilton Fairley.

(1) Weil's disease—three cases, one of which did not show jaundice.

(2) Cases of tropical sprue.

(3) Pathological specimens of acute ulcerative colitis with polyposis.

Dr. N. Hamilton Fairley and Mr. A. H. McIndoe.

Cases of hydatid disease of the liver.

Prof. R. T. Leiper.

Proliferating coenurosis in an experimentally infected gerbille.

The cysts show evagination of the scolices. Many of the evaginations, however, do not produce scolices but appear to become dilated to form daughter

cysts which in turn produce secondary evaginations. The gerbille is not a normal intermediate host of *Multiceps glomeratus*. The cysts which occur in nature in mice do not show these exogenous growths and they normally occur in the subcutaneous tissues, not in the peritoneum as in this instance.

Dr. G. Carmichael Low.

(1) *Filaria bancrofti* infection (with no clinical symptoms) in an old beriberi case.

(2) A case of lymphuria. The condition developed two months after successful treatment for sprue. Cystoscopic examination showed absence of efflux from the left ureter before and after intravenous injection of indigo carmine. Though no filarial embryos were found it appeared that there was some lesion in the lymphatics of the left kidney or ureter.

(3) A case of climatic bubo of about six weeks' duration.

(4) A case, in which cysts of *Entamoeba histolytica* were present in the stools. Suffered from severe pain over a considerably enlarged liver, pain in the right shoulder and fever. A cure was brought about by emetine injections on four days followed by emetine by the mouth.

(5) A case of creeping eruption successfully treated by local applications of oil of chenopodium and freezing with ethyl chloride spray.

(6) A case simulating goundou in a person who had never left England and whose serum gave a three plus positive Wassermann reaction.

Dr. P. Manson-Bahr.

(1) A case of osteitis with periosteal nodules in a Negro seaman from Zanzibar.

Both tibiae (sabre-shaped) as well as arm bones were affected and there was also osteoporosis of the vault of the skull and gummatous ulceration of the ankles. Both Wassermann and Kahn reactions were strongly positive. From the point of view of diagnosis (yaws and syphilis) radiographs of the affected bones were shown as well as lantern slides of yaws osteitis and " boomerang leg " in different native races.

As a contrast a case of osteitis deformans (Paget's disease) in a British seaman of 62 was exhibited which showed definite joint changes. There had been great improvement on combined treatment with calcium and radiostol.

(2) A spleen weighing 2¾ lbs. from a case of splenic anaemia, together with photographs of the patient.

This man came from Rhodesia and after a series of violent haematemeses he developed ascites. Altogether 41 pints of ascitic fluid had been withdrawn. Subsequent to splenectomy in February, 1936, he had made a remarkable recovery and the ascites had disappeared. A number of accidents, including a subphrenic abscess and several large haemorrhages, had made the case more complicated. He had received altogether 29 blood transfusions.

(3) Two cases of leprosy (Hansen's bacillus).

(*a*) A man of 50 years with generalized nodular and macula-anaesthetic leprosy, with thickening of both ulnar nerves, had been under treatment for over two years. He had received three separate protein-shock treatments together with injections of ethyl-esters of hydnocarpus. This case was shown last year, since when there has been so much further and remarkable improvement in his general condition that it is now difficult to recognize that the man has ever been affected.

(*b*) A case of involvement of the right ulnar and peroneal nerves with Hansen's bacillus. This condition with typical anaesthesia developed 12 years after retirement from India. Great improvement in function of the hand (and also restoration of sensation) as well as of the foot, has ensued after three protein shocks. Having been found to be immune to T.A.B. vaccine, a reaction was obtained with intravenous injections of 50, 100 and 200 million *B. coli* in a preparation known as " pyrifer."

(4) A case of filariasis (*W. bancrofti*) with haematochyluria (with 2 per cent. fat in urine) in a Lascar seaman from Calcutta admitted with haematuria.

Inguinal and epitrochlear glands were enlarged. Nocturnal periodic microfilariae, twenty-four per 20 c.mm. Cystoscopy showed the bladder to be normal in appearance, save for small pale yellow vesicles about the size of millet seeds situated on the trigone below left ureteric orifice and it was from these small varicosities that the chyle was leaking into the urine.

(5) A method of treatment of *Taenia saginata* infections which has been found successful in three consecutive cases.

(6) A case of paralysis (diffuse transverse myelitis) following anti-rabies inoculation in a tea-planter, aged 34, from Assam.

On 2nd April, 1936, he inspected a sick horse which was salivating and the animal died 7 days later. On 6th April he was vaccinated against smallpox. On 13th April, anti-rabic inoculation commenced with rabies vaccine—seven daily injections of 4 c.c. each ; after 7th day abdominal wall became very sore. On 30th April he had fever, headache and photophobia ; next day twitching and body pains. On 23rd April flaccid paralysis, right spreading later to left leg. Ascending paralysis as far as umbilicus ; left hand and arm becoming numb as far as the elbow. For a time was semi-conscious and on recovery flaccid paralysis with loss of sphincter control was noted. On admission on 9th July, 1936, spastic paralysis of both legs, positive double Babinski and impairment of sensation up to fifth, thoracic segment.

Diagnosis : Diffuse transverse myelitis.

Proceedings of an **Ordinary Meeting** of the Society, held at
Manson House, 26, Portland Place, London, W.1, at
8.15 p.m., on Thursday, 10th December, 1936.
Sir ARTHUR BAGSHAWE, *C.M.G.*, M.B., D.P.H., *President*,
in the Chair.

PAPER.

SOME OBSERVATIONS ON THE EPIDEMIOLOGY OF KALA-AZAR IN THE SUDAN.

BY

SIR ROBERT G. ARCHIBALD, *C.M.G.*, *D.S.O.*, M.D.,[*]

AND

DR. HASSEEB MANSOUR,
Sudan Medical Service.

Since SHEFFIELD NEAVE[†] first recorded in 1904 the existence of kala-azar in the Sudan, endemic areas of the disease have been found, notably in the Kassala and Fung districts bordering the Abyssinian frontier. Sporadic cases have also been recorded in the Nuba Mountains and in the western province of Darfur. Another endemic area has been found in the Kapoeta district, which more recently has come under Sudan administration, and which lies wedged between Abyssinia on the east, and Kenya and Uganda on the south.

The disease has never occurred in epidemic form, as witnessed in Assam and *other parts of India*, but a smouldering fire of infection, with slight periodic increases of incidence in villages, has served to maintain the disease in the endemic areas of the Sudan.

The observations recorded in this paper apply mainly to the Fung and Kapoeta districts where a few cases of espundia have also occurred, but no cases of cutaneous leishmaniasis of the oriental sore type have been found.

CLIMATOLOGY AND SEASONAL INCIDENCE.

A survey of these endemic areas shows that the disease occurs in districts where the *minimum* annual rainfall is 10 inches ; it does not occur in the northern arid areas of the Sudan. Its incidence therefore appears to be related in some way to adequate rainfall and humidity. In the endemic areas the adequate or necessary rainfall occurs between the months of July and October, when it may reach 100 to 200 mm. per month ; during this period the day temperatures are low and the humidity may reach as high as 90 per cent. For the remainder of the year the climate approaches that of the northern arid areas of the Sudan and it would therefore appear reasonable to infer that the seasonal incidence of the disease coincides with the months that produce the adequate rainfall and humidity, *viz.* July to October. Such a short seasonal incidence may account for the absence of epidemics in areas where the disease has been endemic for some years.

INCIDENCE OF THE DISEASE.

Figures from the report of the medical services show that 289 cases were reported in 1934, and 171 cases in 1935 : the decrease in the incidence last year was probably the result of further closer medical supervision, the diagnosis and treatment of ambulant cases, and greater facilities for treatment in village dispensaries. The disease appears to be more common in adults than in children, and more common amongst males than females ; the greater preponderance in males may be due to the fact that the male is more itinerant than the female and that the male population is more accessible to medical inspection than is the female.

The disease occurs in town dwellers as well as in villagers ; cases have occurred among the well-rationed military and police, occupying government quarters, as well as among the indigent ill-nourished natives living in grass huts. In many instances there was a history of previous contact with, or of proximity to, cases of kala-azar, probably during the rainy season when overcrowding in habitations occurs. In other instances, especially among children, a familial incidence was noted. Europeans have rarely contracted the disease.

INCUBATION PERIOD AND MANIFESTATIONS OF THE DISEASE.

It has not been possible to ascertain the incubation period owing to the fact that the disease requires to be fairly well established before a diagnosis can be made. Even the duration of the fever is no guide to the incubation period, as pyrexias of other origin, notably malaria, and schistosomiasis (*Schistosoma mansoni*) occur in these endemic areas of kala-azar. The formol-gel test for the diagnosis of early cases has given disappointing results.

From observations made the size of the spleen appears to depend on the duration and intensity of the fever : a spleen at a level midway between the umbilicus and costal margin usually indicates fever of at least 4 months' duration, and a spleen at a level with the umbilicus is indicative of 6 months' pyrexia.

Clinically, the disease is similar to that described in other countries ; the double rise of temperature in 24 hours is, however, not such a constant feature as recorded by Indian observers.

The pathology of the disease and the blood changes are similar to the usual descriptions given in other countries. The diminution or absence of eosinophiles has been of diagnostic value in the Sudan.

The causal parasite, *Leishmania donovani*, morphologically and culturally conforms to the characters of the Indian and Mediterranean strains.

In the Fung district the parasite, for some unaccountable reason, appears to be absent or very scarce in the peripheral blood. A thorough examination of 320 *peripheral* blood films taken from 35 cases in all stages of the disease failed to show leishmania either free or phagocyted. In some of these cases peripheral blood films were taken morning and evening, for three or more, and in one case 14, consecutive days.

Owing to the unfavourable climatic conditions it was not feasible to carry out blood culture, attempts were therefore made to infect animals with peripheral blood. Seven monkeys were inoculated subcutaneously and intraperitoneally with varying quantities (0·038 c.c. to 1 c.c.) citrated peripheral and venous blood taken from six cases of kala-azar. None of the monkeys showed clinical signs of the disease ; they were postmortemed at intervals of 150 to 257 days after inoculation, with negative results as regards parasites in the spleen, liver and bone marrow. In the Kapoeta district parasites were invariably present in the peripheral blood, phagocyted within leucocytes. Spleen smears from these Kapoeta cases showed usually massive infections, the parasites being mainly narrow, actively dividing forms. Contrary to expectation, the disease in Kapoeta was no more severe than in the Fung.

PARASITES IN NASAL SMEARS OF KALA-AZAR CASES.

Nasal smears from twenty-five cases were examined for leishmania which were found in seven cases ; in two of these cases the parasites were present in large numbers lying free in the nasal mucus. Probably a greater number of positive results would have been obtained if more nasal smears from each case had been examined, but the search for leishmania among the bacterial flora in nasal mucus proved too laborious.

In one positive case the viability of the parasites was proved by successful infection of two healthy monkeys following intradermal inoculation and intranasal swabbing. Parasites were found in the enlarged spleens of both monkeys, 60 and 80 days later.

FORKNER and ZIA[*] were the first to record the finding of leishmania in nasal mucus and tonsillar smears of kala-azar cases in China, and they have advanced the hypothesis that infection may occur *via* the nose or tonsils.

As already mentioned, infection has been produced in a monkey by nasal swabbing; in a subsequent experiment infection was produced by spraying the nostrils of a healthy monkey using a scent spray with an emulsion containing leishmania. Typical leishmania were found in the liver and enlarged spleen of the monkey 99 days later. By the use of a scent spray the danger of damaging the nasal mucosa was avoided.

FORKNER and ZIA's hypothesis appeared an attractive one for the Fung endemic areas where parasites could not be found in the peripheral blood, but were found in a few instances in nasal mucus. To substantiate this hypothesis nasal swabs and tonsillar smears were taken from healthy contacts of kala-azar cases but leishmania were never found in the numerous smears examined. Three monkeys, inoculated subcutaneously with emulsions of nasal mucus obtained from three healthy contacts occupying huts with kala-azar cases, remained healthy and when autopsied 330 days after inoculation showed neither splenic enlargement nor parasites in liver, spleen and bone marrow. Thus no evidence was obtained that healthy contacts in close association with kala-azar cases acquired nasal or tonsillar affection with leishmania.

ANIMALS EXAMINED FOR EVIDENCE OF LEISHMANIA INFECTION.

A large number of domestic and wild animals have been examined including dogs, cats, fowls, rats, mice, sheep, goats, squirrels, bats, lizards and geckos; in no instance have leishmania been found in the organs of these animals.

Particular attention has been directed to the organs and skin tissues of the domestic dog but no evidence of the existence of canine kala-azar was obtained.

Owing to outbreaks of rabies in the Fung district the dog population has been considerably reduced by periodic destruction of these animals; in the Kapoeta district dogs are rarely seen. From observations made there appears to be no evidence to incriminate the dog as a reservoir of kala-azar; moreover, it has been found that massive doses of parasites are necessary to infect the dog experimentally.

INSECT SURVEY OF THE ENDEMIC AREAS.

It was hoped that an insect survey of kala-azar huts during and after the rainy season combined with dissections and examination of wild insects collected there might produce evidence as to whether insects played a part as vectors of the disease. In the collection of blood-sucking insects in grass huts the most satisfactory "catches" were obtained by inducing the occupants of huts to sleep under sandfly nets in which a few holes 2 inches square had been cut.

[*] FORKNER, C. E, & ZIA, LILY S. (1934). *J. exp. Med.* lix (4), 491.

Blood-sucking insects by this means had ready access to a meal and could be collected the following morning within the nets. The collections obtained included various species of sandfly (*Phlebotomus papatasii, P. minutus, P. africanus, P. squamipleuris, P. perniciosus*), mosquitoes (*Anopheles gambiae*), bed bugs (*Cimex lectularius* and *C. rotundatus*), jassid bugs, lice (*Pediculus vestimenti*), fowl ticks (*Argas persicus*) ; other collections obtained from animals in these huts included dog-fleas, and ticks. Dissections and examination of these insects were carried out with negative results as regards the presence of developmental or other forms of leishmania. Sandflies collected in the kala-azar wards of Singa and Kapocta hospital also proved negative when dissected. House flies (*Musca domestica*) collected in kala-azar huts as well as species of *Lucilia* were dissected with negative results. Plant-feeding bugs, mainly *Lygaeus militaris* and *Aspongopus vidnatus* were also examined ; the majority of these were infested with herpetomonad flagellates which by inoculation into monkeys were proved to be non-pathogenic.

ANIMAL CONTACT EXPERIMENTS.

As data collected showed that close association between the sick and healthy was an important epidemiological factor, two animal contact experiments were carried out.

Four healthy young monkeys were placed in an insect-proof room beside four older monkeys inoculated intraperitoneally with kala-azar material. The four inoculated monkeys contracted kala-azar and two out of the four healthy monkeys were found to be infected 85 and 87 days after being placed in contact with the inoculated monkeys, typical leishmania being found in their enlarged spleens.

A repetition of this experiment resulted in three out of four healthy monkeys being found infected 104, 104 and 113 days later. No opinion can be expressed as to how the infection was acquired by the healthy monkeys.

CONTROL OF THE DISEASE.

In conclusion, it has to be admitted that the problem of transmission of kala-azar in the Sudan, as in other countries, still awaits solution. In the Sudan man appears to be the only reservoir or host of the parasite and he acquires infection (through an insect agent or otherwise) at a definite period of the year which, in terms of rainfall and humidity, commences in July and extends to October. If this hypothesis (based mainly on the climatology of the disease) be correct it appears justifiable to carry out intensive medical inspections of kala-azar villages during the months of May and June for the purpose of detecting and treating obvious cases of kala-azar as well as the less evident ambulant cases ; these inspections would bring about a decrease in the number of " carry over "

infections from one season to another and so lower the incidence of the disease in the endemic areas of the Sudan.

SUMMARY.

1. Endemic areas of kala-azar exist in the Sudan and these are confined to districts with a minimal annual rainfall of 10 inches ; the disease does not occur in the northern arid areas of the Sudan.

2. In the endemic areas the seasonal incidence corresponds to the months July to October when adequate rainfall and humidity occur.

3. Man appears to be the only host and the close association between the sick and the healthy is an important epidemiological factor.

4. In the Fung district it is rare to find the parasites in the peripheral blood ; in the Kapoeta district parasites are invariably present in the peripheral blood. In seven cases parasites were found in nasal smears out of twenty-five cases examined.

5. The method of transmission of the disease from man to man still awaits solution.

6. Undiagnosed and ambulant cases carry over the infection from one season to another. Intensive medical inspection of endemic villages in May and June would detect such cases and by treatment would lessen the " carry over " and so lower the incidence of the disease for the following season.

DISCUSSION.

Sir Rickard Christophers : I have listened with the greatest pleasure to Sir ROBERT ARCHIBALD's paper, because for a long time one has heard that kala-azar existed in the Sudan though one knew little about the details, but to-night we have had all the facts put very clearly and in a most interesting manner.

Kala-azar is still one of the most mysterious diseases, as it was many years ago when the problem of its method of transmission first received attention. Take for example its distribution. It is very prevalent in eastern India and also in the south of India under entirely different conditions. It may be said that it is not present in the north-west because that part of India is very dry. But if it is merely a question of a moist climate, why is it not common in Bombay and why has the Dutch East Indies no kala-azar. There are clearly definite areas where the disease prevails, *e.g.*, the Mediterranean area and its extension eastwards into Turkestan, the Indian and the Chinese areas. We have heard to-night regarding what is evidently another endemic area, the Sudan.

The first point I felt interested in when I heard of to-night's paper was whether we should hear which type of the disease the Sudan kala-azar most resembled. The Mediterranean type is one form of the disease, the Indian is another ; is the Sudan type the Mediterranean or the Indian ? One point in what we have heard to-night seems to bring it nearest to the Indian disease and

that is the absence of infections in dogs. That has always been a matter to which people's attention has been directed in India, and they have consistently failed to find the canine infection which is so prevalent in the Mediterranean disease. I gather that kala-azar in the Sudan must be similar to the Indian disease with regard to this character and probably therefore to the Indian type in general rather than to the Mediterranean type. An interesting point is that eastern Africa is associated with India in some of its faunistic characters and possibly this extends to disease to some extent also.

I do not know whether Sir ROBERT ARCHIBALD paid special attention to the proboscis of the sandfly when he was making his examinations of these insects. This is a most important matter which has come out recently from the work of Dr. ADLER. ADLER believes that infection of the gut of the sandfly with flagellates is independent of the proboscis infection which does not necessarily follow on, and may be regarded as arising distinct from, the gut infection.

It was a pleasure to me to find Sir ROBERT ARCHIBALD saying a word for the contagious theory, because in the early days of kala-azar investigation there was much to support this view and some facts since elicited, such as the finding of parasites in the nasal mucus, also give it some support. I have always been very struck by some remarks made to me by Colonel GREIG who worked in the early days of typhoid investigation in Germany with Professor KOCH. We now regard enteric as a typical man to man disease, but at that time KOCH and everyone concerned with its epidemiology thought of it as an endemic disease, just as we are impressed by the endemic character of kala-azar. The weight of evidence is, however, greatly in favour of the view that the sandfly is the transmitting agent of kala-azar.

Dr. C. M. Wenyon said that as regards the method of transmission of kala-azar there appeared to be two schools of thought : the one which held that an insect vector was necessary ; and the other, favoured by Sir ROBERT ARCHIBALD and hinted at by Sir RICKARD CHRISTOPHERS, which maintained that the chief, if not the only, method of infection was contaminative, in which the leishmania passed directly from man to man. The discovery of leishmania in urine, faeces and in nasal secretions and the proof that such parasites are alive and capable of infecting susceptible animals might appear to lend support to the latter view. It would seem, however, that the presence of parasites in these excretions was merely the result of a general tendency to heavy leishmania infections of the whole skin and mucosa of the body with the result that any abrasion of these surfaces was followed by escape and discharge of leishmania. These leishmania are incapable of survival outside the body for any length of time so that for infection to occur transference of parasites must be immediate. The parasites could not survive in water and could not withstand drying. The contact experiments with monkeys described by Sir ROBERT ARCHIBALD were difficult to explain on any other grounds than infection by direct contact. A

similar experiment with resultant infection had been carried out many years ago with mice. It seemed doubtful, however, if those experiments reproduced the conditions of contact between infected and healthy human beings.

The evidence in favour of certain species of sandfly being transmitters of the disease seemed overwhelming. It had been shown years ago by ROGERS and others that the leishmania were merely rounded form of leptomonas flagellates which are found nowhere in nature except in the intestine of insects or in certain vertebrates. In many cases leptomonas are pure insect flagellates with no vertebrate host. Thus the leptomonas of fleas passes directly from one flea to another through rounded forms which escape in the faeces and are ingested by the larvae. In the case of the leishmania infections such as kala-azar and oriental sore it is known that the ingestion by appropriate sandflies of a few parasites leads to intense and heavy leptomonas infection of the stomach with the migration forwards of these flagellates to the proboscis from which they have been shown to escape when the sandfly feeds. Quite apart from the fact that now in four instances leishmania infection has been transmitted to hamsters by the bite of experimentally infected sandflies, it seems impossible to believe that the remarkable development which takes place in the sandfly is of no significance in the etiology of kala-azar. Not only has this development been shown to take place in sandflies in all the great centres of the disease—Mediterranean, Trans-caucasian, Indian and Chinese—but in all these areas naturally infected sandflies had been discovered in association with cases of the disease. It seemed hardly possible that the Sudan area would be an exception to this rule ; and it appeared safe to predict that further investigation would show that the same close relation-ship existed there between sandflies and kala-azar. On the subject of skin infections already referred to, it is interesting to note that ADLER's work appears to indicate that sandflies infected themselves by taking up parasites from the skin rather than from those that may happen to be in the blood. Sandflies are much more readily infected when fed on hamsters with a good skin infection. The same appears to be true of dogs which in many cases show no signs of disease though having a heavy infection of leishmania in the skin. The presence of leishmania in the skin is being utilised more and more for purposes of diagnosis. Scrapings are made from the dermis with the minimum of bleeding, and films of these may show leishmania on staining.

From what Sir ROBERT ARCHIBALD has said of kala-azar in the Sudan it is clear that there is offered a very fruitful field for research into the problems connected with the spread of this most interesting disease.

Dr. S. Adler : The theory of transmission of leishmaniasis by contact or con-tamination has been put forward several times. FORKNER and ZIA (1935)* found

*FORKNER, C. E. & ZIA, L. S. (1935). Further studies on kala azar. Leishmania in oral and nasal secretion of patients, and the bearing of this finding on the transmission of the disease. *J. exp. Med.*, lxi (2), 183.

leishmania in the oral and nasal secretion of patients with kala-azar. ADLER and THEODOR (1935)* found that in naturally infected dogs the kidney, bladder, nasal and oral mucosa may be heavily infected. The above findings appear to favour transmission by contact but a closer examination of the facts shows that this type of transmission either does not occur at all in nature or is so rare as to be considered an accident and of no importance for the propagation of the disease.

Any disease of dogs or man naturally transmitted by nasal and oral discharges, urine or faeces would eventually become universal, but in the Mediterranean basin visceral leishmaniasis both in man and dog is strictly limited in its distribution to that of certain sandflies of the *major* group. This is true not only over wide areas but even in very restricted localities. Thus in Catania and Naples few or no cases occur in overcrowded urban slum areas where presumably opportunities for transmission by contact are numerous and sandflies rare, while many cases occur on the outskirts where *Phlebotomus perniciosus* is common. In Canea in Crete the distance between an endemic area and one free from the disease was only 350 m. ; in the former *P. major* was numerous and in the latter extremely rare.

It is not too much to say that in nature the leishmania of man and of dog are as closely bound to the distribution of specific sandflies as malaria is to that of *anopheles*.

Although leishmania are found in nasal discharges, urine and faeces and may be living at the moment of their discharge, their survival for any considerable time outside the body is very doubtful in view of the extreme susceptibility of all forms of leishmania to bacterial contamination. On the other hand the flagellate forms survive indefinitely in appropriate sandflies.

At present little is known of the distribution and bionomics of sandflies in the endemic kala-azar region of the Sudan, but it can be confidently predicted that future investigations will show that here, as elsewhere, there is a close relationship between the distribution of a sandfly of the *major* group and kala-azar, and that this sandfly will be found easy to infect with the local leishmania.

It is doubtful whether transmission of visceral leishmaniasis to its natural hosts, man and dog, could be continued for many passages by direct host to host inoculations even under the most favourable conditions. LAVERAN failed to maintain *Leishmania infantum* in dogs by direct inoculation of infected spleen pulp for more than three passages, and therefore suggested that the disease was more probably of human than of canine origin. Actually it is certain that man is of no importance as a reservoir of *L. infantum* in the Mediterranean basin and that the dog is the main reservoir. LAVERAN's experiment suggests that under natural conditions *L. infantum* maintains its infectivity for dog and man by cyclical transmission through appropriate sandflies.

*ADLER, S. & THEODOR, O. (1935). Further investigations on Mediterranean kala azar. *Proc. R. Soc.* B., cxvi, 494.

Colonel J. A. Sinton : These observations on the distribution and possible method of transmission of kala-azar in the Sudan are very interesting. Sir ROBERT ARCHIBALD again raises the question of transmission by means other than an insect vector. DODDS-PRICE and ROGERS pointed out many years ago that, in Assam, the spread of this disease appeared to depend upon a close association between the sick and the healthy. A similar association is now recorded in the Sudan.

In Assam it was found that, when healthy families were moved away from infected lines into new houses only 300 yards distant, the disease did not spread to them, if no infected individuals were included, in spite of the fact that the sick and the healthy had common water and food supplies, and also mixed with each other daily. This fact is more in favour of transmission by an insect with a very limited power of distribution than of more direct carriage of the disease from person to person.[*]

Dr. ADLER, in his note, has suggested that *Phlebotomus perniciosus*, the suspected vector of kala-azar in the Mediterranean area, will probably be found in the Sudan. Within the last few days, the examination of a number of sandflies collected by Mr. LEWIS in a kala-azar area on the Blue Nile, has revealed a male of this species.

While it is possible that some other method than insect transmission may be responsible for the spread of kala-azar in certain areas, the distribution of the lesions of oriental sore on the exposed parts of the body suggests very strongly carriage by an insect vector in the case of the cutaneous leishmaniasis of the Old World.

The discussion on the epidemiology of kala-azar reminds one that in one bacterial disease at least, plague, there are two recognised methods of transmission : (i) by the flea and (ii) by inhalation of infected material.

It is curious that in the Sudan Sir ROBERT ARCHIBALD has been unable to find any domestic animals infected with either visceral or cutaneous leishmaniasis. This is in contrast to the observations on the Mediterranean littoral and in Central Asia, where both forms are not uncommon in dogs. On the other hand, the findings in the Sudan resemble more closely those reported from India. In the latter country numerous observers have failed to discover visceral leishmaniasis among the domestic animals of kala-azar areas, and only three cases of cutaneous leishmaniasis (canine) have been recorded.

Dr. WENYON has mentioned the occurrence of Leishman-Donovan bodies in large numbers in the skin of dogs infected with visceral leishmaniasis. Recently, in an experiment in India, a human volunteer was inoculated by scarification with material from a case of canine cutaneous leishmaniasis. No lesion could be discovered for over a year, but at the end of 13 months a small papule appeared at the site of inoculation. This was found to contain very numerous leishmania, both by direct smears and by culture. This observation suggests

[*] SINTON, J. A. (1925). *Ind. Jl. Med. Res.*, xii, 719-726.

two questions :—(i) Where and in what state was the parasite during this long period ? (ii) If the parasite can remain latent for so many months, how is one to determine the length of the period of incubation in nature ?

Dr. P. Manson-Bahr referred to serological tests such as the formol-gel reaction for the diagnosis of kala-azar. He had found that reliance could not be placed on it in early cases. In one case in particular from India the reaction only became positive after the fourth relapse. The remarks made by Sir ROBERT ARCHIBALD regarding the influence of climate on the occurrence of kala-azar in the Sudan raised the whole question of the distribution of kala-azar in other centres. It did not appear possible to formulate any general law to govern this distribution. In his experience in the realms of ornithology there were similar unaccountable irregularities in the distribution of birds, and it was evident that much work remained to be done before one would be in a position to understand fully the geographical distribution of such diseases as kala-azar, scarlet fever, measles, smallpox, typhus and many others.

Dr. J. W. Lindsay referred to the distribution of espundia in South America and noted that the disease was common amongst woodmen and their families. MIGONE had investigated the disease in Paraguay and had come to the conclusion that the view of the native who attributed it to the cattle tick, *Amblyomma cajennense*, was correct. He could find no evidence that sandflies were involved.

MIGONE himself had, in 1911, in Asuncion, diagnosed a case of kala-azar in an Italian who had immigrated to Brazil in 1897. It might be noted that during the recent viscerotomy surveys carried out in Brazil, examination of 47,000 samples of liver for evidence of yellow fever infection had revealed leishmania infection in forty-one. It would seem that kala-azar was a disease to be reckoned with in South America, and that it and espundia might be found existing side by side. In the course of the Chaco War, in 1934, soldiers suffering from espundia occupied the Chaco territory, and introduced the disease which, according to local observers, was spread by the ubiquitous cattle tick.

Maj.-Gen. Sir John Megaw : The only point I would bring forward I have been induced to make because I feel that Sir RICKARD CHRISTOPHERS is flirting with his old heresy of the person-to-person conveyance of kala-azar. Some years ago Sir RICKARD raised the point and said he was struck with the similarity in the distribution of typhoid and some other such diseases to that of kala-azar, and I asked him then if he knew of any other diseases conveyed from person to person which had a strictly local distribution. I think it is necessary, in view of the suggestions now being made, that this point should be emphasised. Dr. WENYON has emphasised the biological point of view, he has also referred to Dr. ADLER's remarks about the peculiar distribution of kala-azar, and I want to ask that the latter feature of the disease be given full consideration when the mode of transmission is being discussed.

Air Commodore H. E. Whittingham: I would like to mention the occurrence recently of a case of kala-azar contracted in the Aden Protectorate. This is of interest as the disease has not been reported as occurring there before. The case is that of a British officer who had not been out of the Protectorate for the previous 18 months, although he had served in India 5 years previously. Rainfall is minimal in this district, so that we have an instance of kala-azar being endemic in a dry area. As regards the species of sandfly present in this Protectorate, *Phlebotomus perniciosus* had been found repeatedly among catches sent home for identification from time to time.

Sir Robert Archibald (in reply): Sir RICKARD CHRISTOPHERS raised the point as to whether the Sudan form of the disease is of the Mediterranean or Indian type. In the Sudan, as I have already indicated, it is difficult to infect dogs experimentally while I have never found a dog there naturally infected.

With regard to the proboscis of the sandflies dissected, I had read Dr. ADLER's original paper and had noted his reference to the proboscis. In my dissections particular attention was paid to this matter.

Dr. WENYON raised the point as to the occurrence of leishmania in the skin of man and animals. We carried out examinations of the skins of dogs in five or six cases. In one particular village I excised portions of skin and tissue. These were sectioned and examined but no parasites were found. He also asked the number of sandflies dissected. There were not more than 1,200 of them. As regards his remarks concerning the developmental forms of leishmania in these insects I have always regarded them as evidence of a freak form of development.

However, I still have an open mind on the subject of transmission, but I have felt that the question of parasite development in insects has been over-stressed.

In answer to Colonel SINTON's question on the incubation period, I have very few data to give him; it is so difficult to find early symptoms or signs common to all cases of kala-azar. Monkeys gave an incubation period of 50 to 60 days.

In reference to the formol-gel reaction, I was interested in what Dr. MANSON-BAHR said. In the Sudan we tested this reaction pretty fully and got the best results by the formol-gel used in conjunction with the urea stibamine test.

COMMUNICATIONS.

STUDIES ON THE INTESTINAL PROTOZOA OF MAN IN SYRIA AND LEBANON.

I.—The Incidence of Intestinal Protozoa in Hospital Patients at Beirut.

BY

E. W. DENNIS, M.A., Ph.D..

AND

E. E. LUND, M.A., Ph.D.,

From the Department of Bacteriology and Parasitology and the Hospital Laboratories, School of Medicine, and the Department of Biology of the American University of Beirut, Beirut, Lebanon.

Introduction.

It is widely assumed that man in the Near East is commonly infested with many of the various species of protozoa and helminths which are adapted to life in the human intestinal tract. Amoebic colitis is common and the consensus of opinion among practitioners of general medicine within the country, where clinical laboratory facilities are limited, is that the majority of all cases of dysentery which they encounter in general practice are due to *Endamoeba histolytica*. There has been a paucity of information concerning the real incidence of intestinal parasites in this area, and the only previous survey was that by WENYON and O'CONNOR (1917) which was carried out in Egypt under war-time conditions on

troops of the British forces operating in Egypt. The exhaustive study carried out by WENYON and O'CONNOR was exemplary in scope and methods, but the results cannot be assumed to be representative of the condition existing among the populace of the Near East under normal circumstances.

We are now attempting to obtain a reasonably accurate picture of the incidence and distribution of the intestinal protozoa of man in that relatively large portion of the Near East, the French mandated territories comprising greater Syria. The problem has been attacked in two ways : (1) by the study of the intestinal protozoa found in patients at the hospital of the American University of Beirut, and (2) by a systematic survey of the incidence of the intestinal protozoa in the inhabitants of villages of the interior which had been selected for study as typical of the villages existing in the several different natural geographic areas of the country. The present paper deals with a study of the protozoa found in the stools of hospital patients at Beirut. Data on the second portion of the study are under preparation for publication in a subsequent communication. Data on the incidence and distribution of intestinal helminths and on hydatid disease in Syria have previously been reported from our laboratories (YENIKOMSHIAN and BERBERIAN, 1933 ; and TURNER, DENNIS and KASSIS, 1936).

MATERIAL AND METHODS.

The data presented below have been obtained by the examination of 7,229 stool specimens from 4,234 patients in the hospital of the American University of Beirut during the period from July 1st, 1931 to June 30th, 1936. Except for 1931, data are lacking for the summer months of July, August and September of each year due to the necessity of closing the hospital during the months when the University was not in session. Pertinent sections of the clinical laboratory services of the hospital have been integrated with the Department of Bacteriology and Parasitology, and the stool examinations were carried out in this department by skilled technicians under the personal supervision of the head of the department (E.W.D.).

Of the 4,234 patients examined, 3,696 were hospital in-patients. For comparison with this group we have included the data obtained from 538 outpatients who attended the clinics during 1934-1935 (October to June). Most of our material has come from the services of the Division of Internal Medicine and must be considered as having passed through a relatively high degree of selection, although during the latter two years included in the survey the routine submission of stool specimens to the laboratory by the other services became more frequent. This " dilution " of the more selected type of cases by routine material must be considered when evaluating the figures obtained. The number of examinations per patient has fallen far short of the desired three specimens from each patient, the average number of examinations per patient having been 1·71.

The examination of stools for intestinal protozoa is routinely carried out in our laboratories by the study of (1) fresh unstained preparations, primarily for the detection of motile trophozoites, (2) by the iodin-eosin method of KOFOID, KORNHAUSER and SWEZEY (1917) and (3) by a thorough microscopical examination of at least two permanent slides prepared by the fixation of thin smears in Schaudinn's alcohol-sublimate and subsequent staining with Heidenhain's iron-haematoxylin. Culture methods have not been used for routine examinations; although they are useful in certain cases, we have not found them superior to a thorough microscopic examination of stained preparations by an adequately trained technician.

RESULTS.

The results of the analysis of data obtained by the examination of the stools of 3,696 in-patients and 538 out-patients are presented in tabular form in Table I. An average of 1·71 examinations per patient gave the following incidences: *Endamoeba histolytica* 8·43 per cent.; *E. coli* 20·3 per cent.; *Endolimax nana* 13·9 per cent.; *Iodamoeba bütschlii* 5·51 per cent.; *Giardia lamblia* 5·48 per cent.; *Chilomastix mesnili* 5·89 per cent.; *Trichomonas hominis* 0·66 per cent. We have seen *Pentatrichomonas ardenteili* on but two occasions during the past five years.

The data obtained from the hospital patients and those obtained from the out-patients were analyzed separately. The number of patients who were examined several times is indicated in Table I. Although relatively few patients were examined more than five times, certain individuals have been examined as many as eighteen times before the detection of *E. histolytica* confirmed a clinical diagnosis of chronic amoebiasis.

In examining Table I the reader will note that each column consists of two sets of figures. The upper figures, to which we have just been referring, represent actual findings while the lower set have been corrected on the basis of three examinations. The adjusted figures were calculated from the numbers found positive on the first examination (or, as the case may be, the only examination) as compared to the total number found positive in all cases with two or more examinations. Thus of 161 cases of *E. histolytica* which had been examined two or more times, 109 (67·75 per cent.) were positive on the first examination, 41 (25·42 per cent.) were negative on the first but positive on the second examination, while 11 (6·83 per cent.) were not positive until the third examination. Correction was then applied to the number of positive cases where there had been but a single examination. Thus 314 (total positive for *E. histolytica*) minus 161 (positives with more than one examination) = 153 cases positive for *E. histolytica* where only one examination had been made; and it was assumed that these 153 cases represented only 67·75 per cent. of the probable number of positives, hence the adjusted figure was raised to 161 + 153/·6775, *i.e.*, 161 + 226, yielding the figure of 387 (10·5 per cent.) as the probable incidence of

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TABLE I.

THE INCIDENCE OF INTESTINAL PROTOZOA IN PATIENTS OF THE HOSPITAL AND CLINICS OF
THE AMERICAN UNIVERSITY OF BEIRUT.

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		In-patients.		Out-patients.		Total.	
		Numbers.	Per cent.	Numbers.	Per cent.	Numbers.	Per cent.
Patients		3,696		538		4,234	
Total Examinations		6,125		1,104		7,229	
	1	2,378		190			
	2	716		138			
Number of times	3	340		203			
examined	4	114		6			
	5	148		1			
Average number of examinations per patient		1·66		2·05		1·71	
Negatives		2,156	58·4	273	50·7	2,429	57·5
Endamoeba	Actual	314	8·5	43	8·0	357	8·43
histolytica	Adjusted	387	10·5	54	10·0		
Endamoeba	Actual	729	19·7	131	24·4	860	20·3
coli	Adjusted	866	23·4	151	28·0		
Endolimax	Actual	441	11·9	148	27·3	589	13·9
nana	Adjusted	593	16·0	166	30·8		
Iodamoeba	Actual	193	5·22	40	7·44	233	5·51
bütschlii	Adjusted	252	6·8	58	10·8		
Giardia	Actual	203	5·5	29	5·39	232	5·48
lamblia	Adjusted	257	6·9	31	5·8		
Chilomastix	Actual	206	5·73	43	8·0	249	5·89
mesnili	Adjusted	291	7·9	46	8·5		
Tricho-	Actual	23	0·62	5	0·93	28	0·66
monas hominis	Adjusted			Negligible			

E. histolytica in that group of patients. While these figures merely yield an approximation, the percentage of cases which were positive on the first (or only) examination remained moderately constant throughout the 5 years, and the constancy was remarkable considering the small numbers used for yearly calculations in the case of some species. The yearly percentages for *E. histolytica* (first examination) were as follows : 79·0, 65·5, 61·0, 78·1 and 65·0.

In comparing the data from the two sources (*i.e.* in-patients and out-patients) it should be borne in mind that the greater portion of the patients were from the internal medicine service, and that the out-patients, with but few exceptions, were attending the clinic service devoted to the care of gastro-intestinal disturbances. Thus in many respects the two groups are not dissimilar and the incidence of 8·5 per cent. of *E. histolytica* among hospital in-patients as compared with an incidence of 8·0 per cent. for this organism among the clinic patients illustrates this point in a striking manner. However, when the incidences of the non-pathogenic species of protozoa in the two groups are compared there are some marked divergences. *E. coli* occurred in 19·7 per cent. of the in-patients as compared with 24·4 per cent. of the out-patients. The adjusted figures (23·4 per cent. and 28·0 per cent. respectively) show that this difference is real and not an artifact due to the difference in the number of patients in the two groups. The greatest difference between the two groups was in the incidence of *E. nana* which occurred in 27·3 per cent. of the out-patients as compared with only 11·9 per cent. of the in-patients. The incidences of *I. bütschlii*, *G. lamblia*, *C. mesnili*, and *T. hominis* were comparable in the two groups of patients.

The incidence of the various species of intestinal protozoa among hospital in-patients during each of the 5 years included in the survey is shown in Table II. This table shows the annual increase in the number of patients examined. The decrease in 1935-36 was due to necessary curtailment of the amount of " routine " work. On the whole, the figures for the 3-year period 1933-36 are considered more accurate indices of the incidence of the various species among all types of hospital in-patients because of the greater use of the laboratory by services other than that of internal medicine.

The data in Table II show clearly that there was a sharp increase in the incidence of acute amoebiasis due to *E. histolytica* in 1932-33 and 1933-34, followed by a sharp decrease in the incidence of *E. histolytica* from 13·5 per cent. to 4·0 per cent. in 1934–35. Probably this decrease is in small part to be accounted for by a relative decrease in the selection of patients. However, the data on the out-patients were obtained at this same period (1934-35) and an incidence of 8·0 per cent. for *E. histolytica* among this group indicates that the marked decrease in the number of times this organism was encountered among in-patients may be due to a decrease in the severity of the infection rather than to such a very great decrease in actual incidence of *E. histolytica* in the population from which our patients were drawn. In this connection we should point out

that although we have presented the data on out-patients for the year 1934-35 only (because species other than *E. histolytica* had not been recorded in previous years), the incidence of *E. histolytica* in this group during other years has been approximately the same from year to year, and the data presented may be considered as typical.

Also, in considering Table II, it is of interest that *E. nana* increased at the time that *E. histolytica* decreased in incidence among hospital patients. We are certain that this apparent shift in fauna was not due to confusion in the identification of the two species, but we are at a loss for an explanation of the sudden increase in the incidence of *E. nana* while the incidences of other species of intestinal protozoa remained essentially constant during this period. Table II suggests that the incidence of *E. coli* is increasing slightly in the Beirut area.

TABLE II.

INCIDENCE OF INTESTINAL PROTOZOA IN HOSPITAL PATIENTS, PRESENTED BY YEARS.

	Number of Patients.	Number of Examinations.	E. histolytica	E. coli.	E. nana.	Ioda-mocba.	Giardia.	Chilomastix.	Trichomonas.
1931-32	321	483	8·1	13·7	6·2	3·4	4·4	6·0	0·3
1932-33	454	615	13·7	19·0	7·3	8·2	5·5	6·8	2·4
1933-34	998	1,798	13·5	18·3	9·5	5·9	6·0	5·0	1·3
1934-35	1,042	1,794	4·0	20·3	14·5	4·4	5·5	6·1	0·4
1935-36	881	1,435	5·6	22·8	16·0	4·5	5·4	5·1	0·5

Also, it is suggestive that *I. bütschlii* was the only species of the amoebae the incidence of which tended to fluctuate with the incidence of *E. histolytica*.

Although the flagellates *C. mesnili* and *T. hominis* were encountered more frequently during the years in which amoebic colitis was most common, the analysis of the associations of species, as presented in Table III, shows that there was no constant relationship between the incidence of these flagellates and the incidence of *E. histolytica*.

The tendency of certain organisms to occur together is illustrated in Table III, which presents the results of an analysis of the records of 3,219 patients, and shows the number of times each of the species of protozoa recorded from a single host was found alone or in association with one or more different species. In this analysis we were interested in detecting any tendency for a given species to occur in significant association with one or more different species, rather than merely showing multiplicity of infestation.

It was not uncommon to find three or more kinds of protozoa in the same

TABLE III.

FREQUENCY WITH WHICH EACH OF THE SPECIES OF INTESTINAL PROTOZOA WAS ENCOUNTERED ALONE OR IN ASSOCIATION WITH OTHER SPECIES.

Total Number of Cases : 3,219.

Total Number of Negative Cases : 1,872 (58·15 per cent.).

	E. histolytica.	E. coli.	E. nana.	Iodamoeba.	Giardia.	Chilomastix.	Trichomonas.	Number of Individuals in which Organism was encountered.	Per cent. Incidence.
Endamoeba histolytica	134 / 0·764	88 / 1·70	48 / 1·58	39 / 2·64	21 / 1·26	23 / 1·43	9 / 0·985	282	8·76
Endamoeba coli		321 / 0·795	222 / 3·36	80 / 2·52	44 / 1·27	69 / 2·04	3 / 0·526	594	18·45
Endolimax nana			172 / 0·74	49 / 2·55	22 / 1·06	39 / 1·9	4 / 1·16	363	11·3
Iodamoeba bütschlii				58 / 0·56	11 / 1·1	12 / 1·24	3 / 1·83	172	5·35
Giardia lamblia					126 / 1·09	15 / 1·4	4 / 2·23	187	5·81
Chilomastix mesnili						84 / 0·75	5 / 2·83	184	5·72
Trichomonas hominis							19 / 1·04	31	0·96

stool, although five species was the maximum recorded for one host.   Since any attempt to study the frequency of the occurrence of associations involving several species immediately becomes too involved to permit an intelligible expression of the results in tabular or graphic form, we have dealt only with two-species associations.   Cases in which more than two species were present, *e.g.* a case where the patient harboured *E. histolytica*, *E. coli*, and *E. nana*, was recorded once for the *E. histolytica—E. coli* combination, once for the *E. histolytica—E. nana* combination, and once for the combination of *E. coli—E. nana*. Since this method of multiple tabulation would make the total number of times an organism was recorded in all of its associations exceed the number of times that organism was actually encountered, the " totals " shown in Table III indicate the number of patients in which each organism was found, rather than the number of times it was tabulated in the corresponding horizontal column.

In each square of the " checker-board " of Table III we have presented two sets of figures.   The upper figure in each square indicates the number of times the given combination of two species occurred, regardless of the presence of additional associates.   To facilitate the interpretation of these data, another method of expressing the relationships was devised and the results presented as the lower set of figures in each square.   The latter figures represent the ratios of the actual findings (upper figures) to theoretical expectations based on the assumption that the calculated percentage of incidence for each of the two species concerned was distributed through the group of infected hosts purely by chance, *i.e.* by " independent assortment."   For example : *Endamoeba histolytica* occurred in 8·76 per cent., and *E. coli* in 18·45 per cent. of the 3,219 patients.   If these positive cases were distributed by chance alone, 8·76 per cent. of 18·45, or 1·616 per cent. of the total number of patients, would have both organisms.   However, instead of the expected 52 cases positive for both organisms, 88 patients actually showed the *E. histolytica—E. coli* association, and this relationship has been expressed as the ratio of actual over " expected " finding which was 88/52 or 1·70.

To calculate the same ratio for single-species infections the method differed somewhat from the above, but the same assumptions were, of course, maintained.   Table III shows that 1,872 of the 3,219 cases under consideration were negative.   Of the 1,347 cases positive for protozoa, 134 harboured *E. histolytica* alone.   It follows, then, that 1,872 plus 134, or 2,006 (62·32 per cent.), were negative for all organisms other than *E. histolytica*.   If it is assumed, as above, that chance alone operated in the distribution of *E. histolytica*, 62·32 per cent. of the 282 cases found positive for this organism should have been negative for all other organisms ; that is, *E. histolytica* should have occurred alone in 175·74 cases.   However, *E. histolytica* occurred alone in only 134 patients, and the ratio was expressed as 134/175·74 or 0·764.

It has been estimated from the deviations shown by the ratios derived as just described that a ratio between 1·20 and 1·50 suggests nothing of significance

in the relationship of any two of the intestinal protozoa ; likewise a ratio within the range of 0·70 to 0·85 would seem to be without significance for the single-species infections. However, certain relationships recorded in Table III attract attention. As might be expected if it is assumed that more closely related forms tend to thrive best under somewhat similar circumstances, the amoebae, with ratios of 1·58 to 3·36 tend to be associated with each other far more frequently than they do with the flagellates (0·526 to 2·04), and the flagellates likewise tend to be associated with each other more frequently (1·4 to 2·83) than with the amoebae (0·526 to 2·04). Furthermore the following significant points have been brought out :

1. *I. bütschlii* appears to thrive much better when other amoebae are present than it does alone ; none of the other species of intestinal amoebae observed shows a comparable tendency toward association.

2. *E. nana* and *E. coli* occur together more frequently (by ratio) than any other two organisms, the ratio of 3·36 being conspicuously high.

3. *G. lamblia* is an unusually independent organism, appearing alone as frequently as the theoretical value based on " independent assortment " would indicate. The same independence also appears to be characteristic of *T. hominis*, but our cases were too few to be of statistical value.

4. There is no evidence that infection with *E. histolytica* renders a subject more susceptible to infection with other species of protozoa than would a primary infection with any one of the other species.

CLINICAL MANIFESTATIONS.

The hospital records of patients whose stools were examined in our laboratory were referred to in search of information on the nature and severity of symptoms attributable to infection by intestinal protozoa. Naturally our interest centred about those patients who had been positive for *E. histolytica*, but other points of interest were noted. It is not necessary for us to describe the clinical characteristics of amoebiasis, an account of which may be found in any good text-book of tropical medicine. We have arbitrarily classified the patients who were positive for *E. histolytica* according to the presence and severity of the classical manifestations of infection with this organism.

Until there is uncontrovertible evidence to the contrary, we take the attitude that *E. histolytica* in the intestine must always be considered as a pathogen. For this reason we have classified all cases which were positive for *E. histolytica* on the basis of the presence or absence of clinical symptoms. All cases which were positive for *E. histolytica* but which showed no clinical symptoms of amoebiasis were designated as *latent*. All cases which were positive for *E. histolytica* and which manifested symptoms of amoebic colitis or hepatitis, either acute or chronic, were designated as *active*. The active cases of amoebiasis were further classified, according to the severity and duration of the symptoms,

as being either *acute* or *subacute*. When all cases considered were segregated on the basis of these criteria, 89·4 per cent. were classified as *active*, while only 10·6 per cent. were *latent*. All of the latter group were detected in the course of " routine " examinations, and the patients comprising it had been hospitalized for the treatment of conditions which were not attributable to amoebic infection.

The results of our study of the clinical manifestations of *E. histolytica*

TABLE IV.

CLINICAL MANIFESTATIONS OF *E. histolytica* INFECTIONS.
TOTAL CASES OF *E. histolytica* 314 ; INCIDENCE 8·5 PER CENT.

	1931-32.	1932-33.	1933-34.	1934-35.	1935-36.	5 Year Totals.	
						Cases.	Per cent.
Annual Incidence	8·1 per cent.	13·7 per cent.	13·5 per cent.	4·0 per cent.	5·6 per cent.		
Number of Cases	26	63	135	41	49		
Acute	50·0 per cent.	31·8 per cent.	56·5 per cent.	19·3 per cent.	14·2 per cent.	122	38·8
Subacute	45·0 per cent.	59·1 per cent.	36·2 per cent.	67·7 per cent.	64·4 per cent.	159	50·6
Total Active	95	90·9	92·7	87	78·6	281	80·4
Latent	5·0 per cent.	9·1 per cent.	7·3 per cent.	13·0 per cent.	21·4 per cent.	33	10·6
Amoebic abscess in in liver	40·0 per cent.	4·5 per cent.	10·8 per cent.	6·1 per cent.	3·6 per cent.	41	12·1
Amoebic hepatitis	5·0 per cent.	0	2·9 per cent.	3·2 per cent.	17·8 per cent.	17	5·4
Amoebic abscesses, stools negative	20·0 per cent.		6·8 per cent.	6·1 per cent.		22	7·0
Amoebic appendicitis		4·5 per cent.	1·45 per cent.	6·5 per cent.	7·1 per cent.	11	3·5

infection as it has been encountered in our hospital during the past 5 years are presented in Table IV. The table is self-explanatory, and should be of interest to those who are particularly concerned with amoebiasis. Consideration of the yearly data shows a significant degree of correlation between the incidence of acute amoebic dysenteries and the number of cases of amoebic abscess of the liver encountered in any one year ; the occurrence of liver abscesses in 40 per cent.

of the positive cases in 1931-32 is very high. It is worth noting that th
number of cases of amoebic hepatitis, without distinguishable abscess formation
tended to increase during the years when the number of subacute and later
cases was in ascendancy. Also, it will be of interest to the clinician and clinical
laboratory diagnostician to note that no less than twenty-two cases of amoebic
abscess of the liver were encountered in which the stool examinations wer
negative for *E. histolytica*. It is probable that a large number of stool examina
tions per patient would eventually yield a positive result in such cases and
the necessity of numerous examinations should be emphasized in the interest o
accurate pre-operative differential diagnoses. Likewise, from 1 to 2 per cen
of the cases clinically diagnosed and successfully treated as amoebiasis hav
persistently yielded negative laboratory examinations.

Amoebic abscesses of the lungs were occasionally encountered where ther
had been perforation of the diaphragm by abscesses of the liver. Complieation
of this type occurred in less than 1 per cent. of our cases of amoebiasis.

In our presentation of the data in Table II we suggested that the shar
decrease in the incidence of *E. histolytica* among in-patients during 1934-35 a
compared with the two preceding years might be attributable to a decrease i
the severity of the infections, thereby leading to fewer hospitalizations. Thi
suggestion is supported by the clinical data at hand in Table IV. During th
year 1933-34, when the incidence of *E. histolytica* was 13·5 per cent., no les
than 56·5 per cent. of the cases positive for this organism were cases of *acut
amoebic colitis*. The following year, when the incidence of *E. histolytica* wa
only 4 per cent., the percentage of cases which were acute dropped sharply t
19·3. Concurrent with the increase in the proportion of subacute cases and dro
in incidence, the percentage of latent cases increased from 7·3 per cent. to 13
and 21·4 per cent. respectively during the past 3 years.

Of additional clinical interest was the finding of *Giardia lamblia* in cases o
acute duodenitis in adults in whom no other significant organism, either
protozoa or bacteria, could be detected by repeated examinations, and giardi
was the only organism reported for the cases of acute duodenitis of which we
found records.

Table V is given to facilitate comparison of our data on the incidence o
intestinal protozoa with those given in other reports on studies of a comparable
nature.

Finally, in order to ascertain the reliability of our results, which are based
on an average of only 1·71 examinations per patient, we have made an effort
to determine the relationship between positive findings and the number of
specimens examined per patient. For this purpose we studied the detectability
of each species separately and now present the results obtained for three of the
species, *E. histolytica*, *E. coli*, and *G. lamblia* in Graphs A, B and C (p. 420).
It will be noted that the relative number of new positive cases decreased sharply
from the first to the second examination, somewhat less so from the second to

TABLE V.

COMPARISON OF PRESENT FINDINGS WITH THOSE OF OTHER AUTHORS.

Clinic or Hospital Surveys.	Number of cases.	Examinations per c. se.	Total positive for protozoa.	E. histolytica.	E. coli.	E. nana.	Iodamoeba.	Giardia.	Chilomastix.	Trichomonas.
Present Study, American University, Beirut, Syria	4,234	1·71	42·5	8·43	20·3	13·9	5·51	5·48	5·89	0·66
Wenyon and O'Connor (1917), Egypt	328 / 961	1 / 1(?)	.	6·4 / 3·2	31·7 / 10·4	3·0	2(?) / 0·3	5·4 / 6·0	0·67	3·0
Carter, Mackinnon, Matthews and Smith (1917), Liverpool, Eng.	826		50·8	11·4	33·2			18·3	4·4	0·8
Faust, E. C. (1929), Peking, China	13,017	3·07		15·2	20·7	21·6	4·6	1·77	3·9	5·02
Kessel and Mason (1930), Los Angeles, Calif., U.S.A.	2,731	3·0		9·8	18·6	16·9	3·2	4·4	8·3	5·9
Andrews and Paulson (1934), Baltimore, Md., U.S.A.	312	1	11·8	0·3	4·8	2·9	1·6	3·5	1·0	1·6

HINSHAW and SHOWERS (1934), Philadelphia, Pa, U.S.A.	368	1·5	46·2	1·5	4·9	17·4	1·1	2·4	1·4	1·1
MAGATH and WARD (1928), Rochester, Minn., U.S.A.	457	1·75	35	7·9	13·3	13·3	0·9	2·8	7·8	7·4
More General Surveys.										
WENYON and O'CONNOR (1917), Healthy Troops, Egypt	1,979	1		5·3	20·0	0·5	3·0	4·8		1·1
MELENEY, BISHOP and LEATHERS (1932), Tennessee, U.S.A.	20,237	1		11·4	32·1	11·9	4·1	14·7	2·9	
TER-MATEVOSSIAN (1933), Armenia, U.S.S.R.	1,200			13·8	32·0	12·0	14·3	8·2	7·0	15·0
				20·6	57·0	17·4	22·3	24·0	13·8	31·5
ANDREWS, J. (1934), Fresnillo, Mexico	2,303	1	76·9	12·9	61·1	26·0	15·7	5·0	7·7	15·8

Except for the first two columns of data all figures represent percentages.

the third, and after the third examination the remaining number of undetected positives was small. In only one case out of 203 which were positive for giardia did that organism remain undetected until the fifth examination. As is indicated

Endamoeba histolytica

GRAPH A.

Endomoeba coli
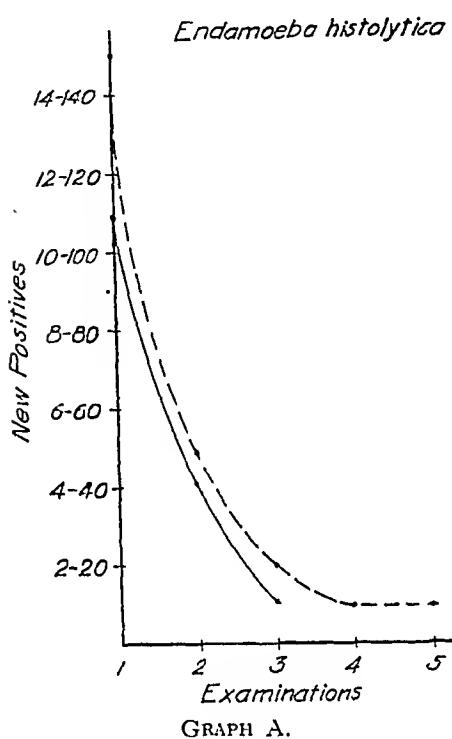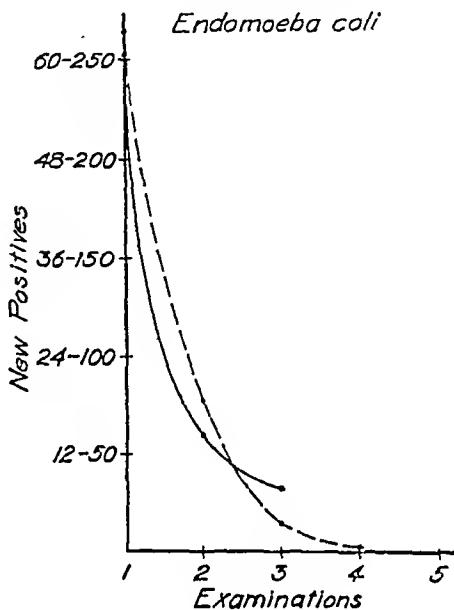
GRAPH B.
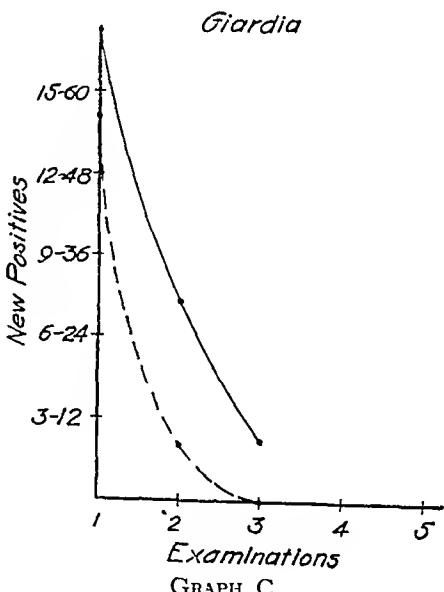
Giardia

GRAPH C.

GRAPH A.—Detectability of *E. histolytica* with reference to the number of examinations required for its demonstration in each positive case.

GRAPH B.—Detectability of *E. coli*.

GRAPH C.—Detectability of *Giardia lamblia*.

NOTE: In each of the three graphs the larger numbers in the vertical column and the solid line refer to data on hospital patients; the smaller numbers and interrupted line refer to the out-patient group. The figures in the vertical column indicate the number of positive cases in the two series.

in Graph A, three examinations revealed at least 92 per cent. of the cases which were positive for *E. histolytica*.

The curve of detectability of *E. nana* closely resembled that of *E. histolytica*. *I. bütschlii* was found with a little less ease, but its graph indicated that about 85 per cent. of the ultimately positive cases are detected by three examinations. *C. mesnili* was found to be very erratic in its appearance, and it was difficult to even estimate the value of multiple examinations in detecting it. The results as presented in the accompanying graphs warranted the use of three examinations as the basis for adjusting our incidence values as given in Table I.

DISCUSSION AND CONCLUSIONS.

Intestinal protozoa were found in 42·5 per cent. of 4,234 patients of the hospital and clinics of the American University of Beirut. The species of protozoa which were found were *E. histolytica* (8·43 per cent.), *E. coli* (20·3 per cent.), *E. nana* (13·9 per cent.), *I. bütschlii* (5·51 per cent.), *G. lamblia* (5·48 per cent.), *C. mesnili* (5·89 per cent.), and *T. hominis* (0·66 per cent.). *P. ardenteili* was observed in but two cases. *D. fragilis* was not encountered during the 5 years of work reported, hence this amoeba of man must be very rare in Syria and this portion of the Near East.

The results of our study of the tendency of certain species of intestinal protozoa to occur in association with each other suggest that a properly controlled *comparative* study of the influence of environmental factors on the different species would add significant data to our knowledge of the biology of these important micro-organisms.

It is apparent that amoebiasis is an important clinical and public health problem in Beirut and the adjacent Lebanon mountains. Our laboratory data on the bacillary dysenteries have not yet been completely correlated with the clinical diagnoses and histories, so that it is not possible to state with certitude just what proportion of hospitalized dysenteries are amoebic in origin. However, from the data at hand it appears that the proportion is well above 10 per cent.

Since there is no adequate evidence available which indicates that strains of *E. histolytica* differ in virulence, it is worth-while noting certain local abnormal environmental conditions which may have contributed to the increased incidence of amoebiasis and to increased susceptibility of the host during the peak years of 1932-33 and 1933-34. The country suffered severe drouth during the period cited, and although Beirut itself has a fine water supply the mountain villages suffered from shortage of water so that in many instances shallow wells had to be depended upon for domestic requirements. Fruits and vegetables which constitute a large portion of the local diet and provide much of the cash income were unusually scarce. The significance of these factors is increased by the fact that thousands of the residents of Beirut, regardless of financial status, spend most of the summer in the neighbouring mountains to avoid the debilitating heat and humidity of the city.

The influence of the drouth on the fly seasons probably was a more important factor. The ubiquitous filth flies are present at all seasons of the year, but are usually greatly reduced in numbers during the winter months of heavy rains. During the two winters under consideration, flies were abundant at all times. Promiscuous defaecation in lanes, streets and open plots of ground is of common occurrence, the urgencies of nature taking precedence over imposed conventions among the more ignorant elements of the populace. This abundance of exposed faecal material, associated with mild weather and abnormally prolonged fly seasons surely favoured the wave of amoebiasis which was encountered.

Our data indicate that three examinations per patient, if carefully conducted, are adequate for the routine examination of *hospital patients* for the detection of infestation with intestinal protozoa. We would emphasize, however, that if there are clinical indications suggestive of the presence of *E. histolytica* in a patient the number of examinations should be limited only by the circumstances. In one of our cases where there was a question of a differential diagnosis between that of amoebic abscess of the liver or a non-malignant tumour, we were rewarded by the finding of *E. histolytica* in the eighteenth specimen submitted. A diagnosis of probable amoebic abscess was confirmed at operation.

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NEPHRITIS IN THERAPEUTIC MALARIA.

BY

MARY E. TYARS, M.D., B.S. (Lond.), D.P.M.,*

Assistant Medical Officer, Hanwell Mental Hospital ; late Assistant Medical Officer, Horton Mental Hospital, Epsom.

Introduction.

For at least 30 years the incidence of a chronic type of nephritis in cases of quartan malaria has been recognized by workers in tropical countries (Watson, 1904 and 1905 ; James and Gunasekara, 1913). The first cases described were of " hydraemic " type and occurred in patients whose malaria was long continued and of low febrile type, showed frequent relapses, or had passed unrecognized and so had received scant treatment or none at all. In these cases the symptoms were debility, oliguria with dropsy in advanced cases, and albuminuria. A further group of " azotaemic " cases was subsequently recognised, though occurring only rarely (Giglioli, 1930 ; Goldie, 1930), in which there was no dropsy ; but headaches, vomiting, dizziness, cardiovascular complications and albumin with casts in the urine were prominent features. Both types of cases cleared on efficient treatment of the malaria with quinine.

Manson-Bahr and Maybury (1927) described two cases of azotaemic nephritis occurring in quartan malaria and concluded that the association might be purely fortuitous ; but that, if this is not so, the nephritis may be more commonly associated with the excretions of toxins of only one species—*viz.* *P. malariae.* They add that the evidence would have been stronger if backed by blood urea estimations. Goldie (1930), however, formed the conclusion from his observations on reviewing eight years' material (nearly 10,000 cases, of which 23 per cent. were quartan malaria) that albuminuria can be found in any chronic cases of malaria but that it is most likely to appear in quartan, which is the most chronic and protracted form. He found that, where

P. malariae and *P. vivax* prevail so that chronic infections are common, there is much albuminuria, little acute nephritis, and subchronic interstitial nephritis abounds. On the other hand, where *P. falciparum* abounds, albuminuria is also frequent and acute nephritis not rare, but where, as in the Roman Campagna, the fever is seasonal with long respites, both albuminuria and nephritis are rarely found. GOLDIE believes that *P. falciparum* acts on the kidneys by mass action producing an acute condition, whilst *P. vivax* and *P. malariae* produce their effects by continued cumulative action. FAIRLEY and BROMFIELD (1933) have estimated the blood urea in eight patients with benign tertian and twelve cases of malignant tertian malaria by Archer's modification of Folin's method. In two benign tertian cases on which seventeen estimations were made a transient rise above normal was found and related to the accelerated katabolism of fever rather than to nitrogen retention in view of negative clinical findings. A third benign tertian case (cerebral syphilitic) showed an increase to 108 mg. per cent. with oliguria, albuminuria, casts, drowsiness and Cheyne-Stokes breathing. They concluded that the malaria here had " precipitated uraemia in a syphilitic patient with arteriosclerosis and damaged kidneys."

Also WOLSKY and SCHEWELEWA (1930), combining Volhard's and Mosenthal's methods of estimating renal function by urinary output and concentration of urea and chlorides, in seventy-six cases which had had intermittent malaria for some years, found that damage occurred in about 60 per cent. of cases of each type of malaria and that " in many cases the injury is protracted." The injurious factors are deposition of pigments, the results of enormous red blood cell disintegration, toxins, and possibly the hyperaemia induced in the kidneys by the febrile process.

PRESENT OBSERVATIONS.

In view of the observations noted above, and the increasing use of malaria therapy for cases of general paralysis of the insane and kindred disorders, an attempt has been made in this investigation on the patients undergoing malaria treatment at Horton Mental Hospital to ascertain how far kidney damage might be responsible for untoward developments during the fever, and whether the incidence of this complication is greater in cases treated with *P. malariae* than in others.

The hydraemic type of nephritis appears to be rare in therapeutic malaria, and no recent case has occurred amongst the patients treated at Horton, but several have shown symptoms which might be attributed to uraemia (*viz.* headache, drowsiness, dyspnoea, persistent vomiting and convulsions).

Estimations of blood urea and examinations of urine for albumin casts, red blood corpuscles and pus, with measurement of specific gravity, were therefore made on twenty-one cases of quartan malaria, twelve of benign tertian, and two of malignant tertian—before commencement of fever, during fever and

after its termination. The quartan strain of malaria employed was obtained from Professor KIRSCHBAUM of Hamburg; the benign tertian came from an indigenous case in Roumania; and the malignant tertian was also a Roumanian strain.

The estimations of blood urea were made by Archer's modification of Folin's method (ARCHER and ROBB, 1925) which gave a figure for the non-protein nitrogen of the blood found to be accurate to within 5 per cent. Multiplication by the factor 2·143 converted this to the urea value. The normal urea content of the blood was taken as 15 to 45 mg. per cent.

Although tests while under treatment were taken either during a fever peak or in an afebrile phase, the variation did not appear to affect the result, as the ratio of increased to unaffected urea values was approximately the same in both phases.

The age and sex of the patient also do not appear to affect the reaction in any significant way.

It was found that the cases fell into four groups:

1. Those showing a rise in blood urea to above normal levels during treatment.

2. Those showing a rise in blood urea but still remaining within normal limits and returning subsequently to near prefebrile level.

3. Those showing practically no change throughout.

4. Those showing a fall in blood urea during treatment, which fall was maintained, in all save one case (Q11), at the time of the final test.

The eight cases showing marked increase in blood urea, viz. four quartan (19 per cent.), one malignant tertian and three benign tertian (28 per cent.) are set out in Table I (p. 426).

Two of them (Q10 and B8) were known to have some kidney involvement, as shown by the trace of albumin, before treatment commenced. Q10 had mild cyanosis, dyspnoea and rapid pulse of poor volume in the sixth peak, so fever was aborted; but she stood seven more peaks well. B8 had diabetes but was able, in spite of some cardiac embarrassment and vomiting, to stand four short bouts of low fever, making a total of 13 days.

Q3 and B11 had also to be given quinine owing to cardiac and respiratory symptoms but showed none attributable to uraemia. Q6 and M5 who died did show symptoms suggestive of uraemia but M5's blood urea was low. Q12 and B9 reacted to their fever therapy normally.

In Table II are recorded those cases with a moderate rise in blood urea during fever: of these, seven were quartan (33 per cent.) and five benign tertian (36 per cent.). They seem comparable with the cases described by FAIRLEY and BROMFIELD which showed the transient rises due to increased katabolism.

Of our cases, three quartan and two benign tertian had no symptoms which called for extra care; three quartan (Q5, Q13, Q19) and one benign tertian (B13) developed signs of collapse (poor colour, unduly rapid pulse, vomiting, etc.)

TABLE I.

Type of Malaria	Case Number	Age	First Test — Urine before Fever — Blood Urea in mg. per cent.	Interval in Days after Infection	Albumin	Casts	Leuco-cytes or pus	Specific Gravity	Second Test — Blood Urea in mg. per cent.	Condition at Time of Test	Day of Fever	Abnormalities of Urine in Fever — Albumin	Casts or Blood	Leuco-cytes or pus	Specific Gravity	Third Test — Blood Urea in mg. per cent.	Interval in Days after Fever	Urine after Fever — Albumin	Casts	Leuco-cytes or pus	Specific Gravity
Quartan	Q3	53	43	2	Normal	Normal	Normal	1030	62	In fever, 9th peak	14th	Slight trace	—	Present	1010	39	14	Normal	Normal	Normal	
	Q6	51	23·5	14	Normal	Normal	Normal	1030	43	24 hours after 5th peak when cyanosed, and with poor pulse (15th day of fever)	10th / 25th / 29th	Trace / Faint trace / Trace	— / — / —	— / — / +	1020 / 1021 / 1035	98	*On 33rd day of fever	Trace / Faint trace	—	—	1025
	Q10	42	17	12	Trace	—	‡ +	1014	98	Commencing 18th (last) peak	4th	Faint trace	—	+ +	1020	17	13	Faint trace	—	Present	1036
	Q12	53	36·5	15	Normal	Normal	Normal	1020	53·5	In fever, 5th peak	7th	,,	—	Present	1010	21	4	Normal	Normal	Normal	
Benign and Malignant Tertian	M5	49	26	134	Normal	Normal	Normal	1022	122	5 days later, after 3 days' stupor, comatose with Cheyne-Stokes breathing. Died next day	Day of 2nd test	Heavy trace	Few granular casts	+ +	1026			Not obtainable			
	B8	51	32		Trace	—	—	1019	139	In fever, 5th peak	Throughout fever	Variable between faint trace and 2 per cent.	Granular casts intermittently. Hyaline on 1st 5 days. Few R.B.C. 1st and 2nd days	Present intermittently	1006 / 1025			Not obtainable			
	B9	52	30	7	Normal	Normal	Normal	1010	63	In fever, 6th peak			Nil		?	21	7	Normal	Normal	Normal	
	B11	40	30	28 This case was re-infected	Normal	Normal	Normal	1031	70	Descending 4th peak	4th	Faint trace	—	+	?	43	14	Normal	Normal	Normal	

* Test taken 24 hours after start of convulsions. Patient died on 35th day.

‡ Convulsions started 12 hours after this test and lasted 24 hours. Fever was checked at once, and only 2 low peaks ever developed.

TABLE II.

Type of Malaria	Case Number	Age	First Test: Blood Urea mg %	First Test: Interval Days after Infection	Before Fever: Albumin	Before Fever: Casts	Before Fever: Leucocytes or pus	Before Fever: Specific Gravity	Second Test: Blood Urea mg %	Condition at Time of Test	In Fever: Day of Fever	In Fever: Albumin	In Fever: Casts or Blood	In Fever: Leucocytes or pus	In Fever: Specific Gravity	Third Test: Blood Urea mg %	Third Test: Interval Days after Fever	After Fever: Albumin	After Fever: Casts	After Fever: Leucocytes or pus	After Fever: Specific Gravity
Quartan	Q5	49	21	10	—	—	+	1018	30	24 hours after 10th peak	6th 24th 28th	Trace Faint trace	— —	++ —	±1010 1010	23·5	20	Faint trace	—	++	1010
Quartan	Q8	58	30	27	Normal				45	12 hours after 9th peak		Faint trace	—	—	1010	30	9	Normal			
Quartan	Q13	53	39·5	14	Normal			1010	45	48 hours after 4th peak	2nd 7th 14th 22nd	Trace Faint trace „ Trace	— — — —	+ ++	1020? 1028 1027	34	19	Normal			
Quartan	Q14	29	21	14	No report				30	Descending 5th peak			Nil	No report		19	10	No report			
Quartan	Q18	16	21	4	Normal			1018	34	16 hours after 9th peak (Patient died on 23rd day)	12th	Trace	—	Present	1024	21	21				
Quartan	Q19	53	15	13	Normal			1020	34	In fever, 7th peak	3rd 24th	Heavy trace Trace	— —	++ Few	1016 1036	34		Normal			
Quartan	Q21	52	30	9	Normal			1021	36·5	40 hours after 5th peak	1st 2nd	Trace Trace	— —	++ +	? ?	32	14	Normal			
Benign Tertian	B2	41	34	11	Normal			1012	41	In fever last peak	8th	Trace	—	Present	?	19	17	Normal			
Benign Tertian	B6	42	32	6	Normal			1023	45	Descending 10th peak		Trace	Nil		?	21	16	Normal			
Benign Tertian	B10	49	34	7	Normal			?	41	Commencing 3rd peak			Nil	Present	?	23·5	7	Normal			
Benign Tertian	B12	42	21	3	Trace	—	+	1026	43	24 hours after 3rd peak	10th 13th 17th 30th	Trace Trace Faint trace Trace	Blood + Blood ++ Gran. casts +	++ ++ +	1015 1010 1014	26	20	No report			
Benign Tertian	B13	37	26	7	Trace/gran casts pres. + till 1 month before fever; subsequently normal			1010	43	In fever 6th peak	30th	Trace	—	+	1015		21	No report			

and one benign tertian (B10) had convulsions on the 10th day of treatment, but recovered rapidly.

One patient (Q18) died. He was extremely excited throughout treatment but stood the fever apparently quite well until the last peak (10th) soon after which he collapsed suddenly.

B2 had a slight seizure on the 19th day but recovered though remaining in poor condition.

Cases with no change, or a decrease in blood urea are shown in Table III. As the significance of the fall in urea content is not known, these two groups have been combined for consideration.

There were ten quartan, one malignant and four benign tertian cases in this section. The two quartan and the one malignant tertian alone gave no unusual symptoms at all. The rest showed more or less distress when fever was at its height. One case (Q16) died in coma with respiratory difficulty and was thought at first to be similar to Q6 and M5, but eventually gave evidence of lobar pneumonia which was confirmed postmortem.

In addition, B1 had severe seizures, but no increase in blood urea was discovered. He died during another bout of seizures some time after treatment : at postmortem there was considerable increase of intracranial fluid and no evidence of kidney lesions. Convulsive seizures during malarial therapy may therefore not uncommonly be of the type so often met with in general paralysis, and blood or urine examination is useful in establishing their origin.

Another noteworthy case was B4 who had very high blood urea before fever. She showed distress but no definitely nephritic symptoms, and after being rested with quinine completed her treatment satisfactorily.

SUMMARY.

In a series of thirty-five cases of therapeutic malaria 19 per cent. of quartan cases and 25 per cent. of benign tertian showed, during fever, an increase of blood urea to dangerous levels. Not all of these, in fact only two, one quartan and one malignant tertian, showed symptoms described as characteristic of azotaemic nephritis. Both these two patients died and the diagnosis was confirmed postmortem.

Two other quartan cases died during treatment—one of pneumonia and one of cardiovascular disease. In neither case was there any evidence that the kidneys were to blame.

One of the benign tertian cases had convulsions which were attributable to the cerebral condition.

One benign tertian and three quartan cases had high blood urea and four benign tertian and two quartan cases albuminuria before treatment, but all were able to receive a course of malaria therapy without signs of kidney failure.

Type of Malaria	Case Number	First Test: Blood Urea in mg. per cent.	Interval in Days after Infection	Urine before Fever: Albumin	Casts	Leuco-cytes or pus	Specific Gravity	Blood Urea in mg. per cent.	Condition at Time of Test	Second Test: Day of Fever	Abnormalities: Albumin	Casts or Blood	Leuco-cytes or pus	Specific Gravity	Blood Urea in mg. per cent.	Third Test: Interval in Days after Fever	Urine after Fever: Albumin	Casts	Leuco-cytes or pus	Specific Gravity
Quartan	Q1	137	2	Normal	—	Normal	1015	64	36 hours after 4th peak	20th	Faint trace	—	++	1025	61	3	Normal			
	Q2	68	2	Normal		Normal	1018	66	24 hours after 9th peak			Nil			32	2	Normal			
	Q4	30	10	Normal		Normal	1014	21	24 hours after 6th peak			Nil			23.5	10	Normal			
	Q7	28	14	Normal		Normal	1018	21	12 hours after 6th peak			Nil			21	21	Normal			
	Q9	30	16	Normal		Normal	?	32	48 hours after 9th peak			Nil			30	28	Normal			
	Q11	86	9	Variable, trace once —nil	gran. casts once	+	1015–22	47	In fever 3rd peak	3rd; 7th; 23rd; 26th; 29th	Faint trace; Trace; Faint trace	—; Gran. casts +; —	++; ++; ++; +	1010; 1031, 1026, 1020; 1029	88	10	Faint trace	—	+	1015
	Q15	39.5	14	Normal		Normal	1026	28	20 hours after 5th peak	34th	Faint trace	—	+	1025	30	6	Normal			
	Q16	23.5	4	Normal		Normal	1018	23.5	In fever 12th peak (Pt. died 4 days later)	25th	Faint trace	—	+	1019						
	Q17	32	4	Normal		Normal	1019	23.5	12 hours after 11th peak	20th; 23rd; 32nd	Trace; Faint trace	Nil	++	1018 ±1020	23.5	28	Trace	—	+	1010
	Q20	23.5	1	Normal		Normal	1010	21	In fever 6th peak			—; —		26	21	Normal			
Benign and Malignant Tertian	B1	23.5	12 hours after start of seizures (fever starting)	Normal			?		Fever arrested after first seizures owing to cardiac weakness											
	B4	185	3	Normal		Normal	1005	112	In fever 4th peak	12th	Faint trace	—	Present	1019	28	3	Normal			
	B3	39	10	Normal		Normal	1020	36.5	In fever 8th peak	4th; 6th	Faint trace; "	Nil	—; +	1025; 1025	36.5	14	Normal			
	B7	41	5	Trace	—	+	1032	21	2 days after 4th peak		Faint trace; "	Few gran. casts	+; Present	1020; 1022	30	7	No report			
	M14	30	8	Normal		Normal	?	34	In fever 2nd peak	4th; 8th	Trace; Faint		+; Present		28	14	Normal			

CONCLUSIONS.

The numbers are too small to draw conclusions as to the incidence of nephritis in therapeutic malaria, but two cases occurred in a series of thirty-five patients.

When definite nephritic symptoms are not present, a rise of blood urea to above normal levels is often, but not invariably, accompanied by signs calling for a remission of fever. Such signs are found also in patients who show no positive evidence of kidney disfunction.

There is no evidence that the liability to kidney damage is increased in cases treated· with quartan malaria. Having regard to the mode of action of the quartan parasite on the kidneys and the absence of the necessary conditions during therapeutic fever there is no reason to suppose that in Case Q6 the same result would not have occurred equally, or even more readily, had either of the other varieties been employed.

The presence of high blood urea content or albuminuria does not appear to contra-indicate the use of malaria therapy, provided the fever is aborted as soon as the usual symptoms of distress show themselves.

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THE MANIFESTATIONS AND MEASUREMENT OF IMMUNITY TO MALARIA IN DIFFERENT RACES.

BY

D. BAGSTER WILSON, M.D., B.Ch., D.T.M. & H.*

AND

MARGARET E. WILSON, M.B., Ch.B.

From the Malaria Unit, Moshi, Tanganyika.

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I.—INTRODUCTORY.

In attempting to explain the differences which they had observed in the endemic data of two widely differing communities subject to malaria, Swellen-grebel *et al.* (1931) and Schüffner *et al.* (1932) concluded that there was a fundamental difference in the immune mechanisms of the two cases. It is the object of the present paper to discuss, in the light of our own findings and those of other workers which are comparable with them, whether such differences in the

endemic data do in fact represent racial distinctions in the human reaction to malaria, or alternatively how they may be correlated ; and, further, to make some contribution to the general interpretation of observations on the spleen and parasite rates.

Table I summarises the findings of SWELLENGREBEL *et al.* (1931) in South African Bantu, and almost identical results could be obtained in thousands of hyperendemic African villages. They could also be obtained, as we have ourselves found, in villages of the more primitive races in India. Table II, taken from the same authors, summarizes the results found in Malays with whom they were contrasting the Bantu. These results could, however, also be paralleled fairly closely among the Aryan plains-people of India, and even among the Hamitic Masai in East Africa.

While, then, there are similarities to be found in widely separated countries, there are also great contrasts in the same country, and even in contiguous areas with differing physical conditions.

TABLE I.

PARASITE AND SPLEEN RATES IN SOUTH AFRICAN BANTU.

Age Group.	Total Examined.	Spleen Rate.	Subtertian Per cent.	Benign Tertian Per cent.	Quartan Per cent.
0–1	55	62	53	5	4
2–5	114	93	86	19	8
6–10	173	90	68	16	2
11–15	201	76	53	16	1
16–20	234	54	46	9	1
20–40	350	47	36	2	1
Over 40	115	51	21	5	1

II.—SOME PREVIOUS RECORDS BY OTHER WORKERS.

Tables I and II and Fig. 2 (p. 443) are from SWELLENGREBEL *et al.* (1931), and sufficiently summarize their results for the present purpose. It should, however, be noted that the parasite rates given are, in comparison with the other series to be discussed, too high, for each mixed infection is counted twice or three times, depending on the number of species present. They state, however, that the parasite rate rose in the Malays to a maximum of 50 per cent. in the age-group 1 to 5, and fell to 7 to 10 per cent. in adults ; while in Bantu it rose to 100 per cent., and fell to 40 per cent. in adults. They postulate an absolute parasite immunity in the Malay, but a parasite tolerance, or premunition, to reinfection in the case of the Bantu ; this latter state being only obtainable in races which have an innate tolerance to any malarial infection.

TABLE II.

PARASITE AND SPLEEN RATES IN MALAYS AT MANDAILING.

Age Group.	Spleen Rate.	Subtertian Per cent.	Benign Tertian Per cent.	Quartan Per cent.
0–1	87	27	14	9
2–5	93	34	8	16
6–10	92	21	3	9
11–15	94	15	0·9	7
16–20	92	7	0·8	2
20–40	90	6	0	0·7
Over 40	89	7	0·7	1ʹ

But even in Malays, and even in Sumatra, HELFFERICH (1934) has described the " Bantu " type of reaction, in his finding of a low spleen rate in combination with a much higher parasite rate at all ages. This is another example of the contrasts to be found in adjacent territories.

Over the greater part of India malaria approximates to the type described by SCHÜFFNER for Malays. MACDONALD and MAJID (1931) in an investigation round Karnal in the Punjab, found that in villages with spleen rates of 39 per cent., 32 per cent., and 20 per cent. the parasite rates were respectively 14 per cent., 8 per cent. and 10 per cent. in children. Another group of Aryan plains-people has been described by SWEET (1933). His findings in villages in Mysore are summarized in Table III and Fig. 1 (p. 443). The spleen rate is nearly double the parasite rate and maintains a high level at all ages.

TABLE III.

PARASITE AND SPLEEN RATES IN MYSORE.

Age Group.	Parasites.		Spleens.	
	Examined.	Per cent.	Examined.	Per cent.
0–4	448	38·8	376	66·5
5–9	1702	33·1	1688	73·8
10–14	1232	27·8	1196	72·0
15–19	259	28·6	244	74·6
Over 20	704	22·4	593	74·4

The group of non-Aryan children examined by CHRISTOPHERS (1924) in Singhbhum in Orissa is in entire contrast to these other Indian groups. The parasite rate was extremely high, and the Spleen Rate somewhat lower than this. His parasite counts are shown, in Fig. 5 (p. 446), to be almost identical with those found in similarly hyperendemic communities examined by ourselves.

III.—SUMMARIES OF OUR OWN EXAMINATIONS.

Methods.—Spleen examinations were made in the standing position, and measurements recorded by Schüffner's method. The "Average Enlargement" of the spleen shown in our tables below is the mean of the sizes recorded by this method. Where " Spleen Rate " is written thus, *i.e.*, with capitals, it refers to the child rate at age 1 to 10 ; otherwise it refers to the rate at any age.

Thick and thin films were examined in each case, the former being used for counting parasites against leucocytes, and the latter for identification of species. All films were examined by ourselves.

1. MASAI.

In a medical survey of the Masai Province of Kenya, PHILIP (1931) found that in various groups of Masai the following spleen and parasite percentages were present.

TABLE IV.

SPLEEN AND PARASITE RATES IN KENYA MASAI (PHILIP).

Group.	Spleen Rate.		Parasite Rate.	
	Children.	Adults.	Children.	Adults.
A	1·5	7	1	3
B	14	9	6	6
C	35	23	16	13
D	62	32	13	13

These results led us to wonder what the corresponding parasite counts would be, and we examined a series of 320 Masai of all ages, with the results shown in Table V, and in Figs. 1 and 5.

It is evident that the malarial status of these communities closely resembles that of the Indo-Aryan communities already cited. It is unfortunate that all these examinations were made at a time when malarial transmission was either absent or negligible ; but they do show a very low development of immunity, as evidenced by the small reduction with age in the parasite rate, and an even smaller reduction in the parasite count. The Masai recognize, and are very much afraid of, malaria. They are a semi-nomadic, Hamitic tribe, of which most groups live away from places where there is any considerable anopheline

infestation; they do, however, move on to rivers at the end of the dry season, or in exceptionally dry years. It is difficult therefore to make any estimate of their liability to malarial infection.

TABLE V.

SPLEEN AND BLOOD EXAMINATIONS IN TANGANYIKA MASAI.

	Spleens.				Parasites.	
Age Group.	Number Examined.	Per cent. Palpable.	Average Enlargement.	Number Examined.	Per cent. Positive.	Average Count per c.mm.
0–1	12	50		12	25	
1–10	78	26	1·55	78	32	266
11–20	63	44	1·42	64	34	196
Over 20	149	41	1·48	165	24	117

2. DIGO.

A full account has already been given in these TRANSACTIONS, WILSON (1936), of the natural history of malaria in some villages of this typical Bantu tribe; but, for convenience of reference and comparison, some of our findings in children are here summarized in Tables VI and VII, and shown graphically in Figs. 4 and 5. We should like also to draw attention to some other relevant observations.

Within the first six months of life all babies acquire as intense an infection as any other non-immune (our experiences have been confined to Indians, Europeans and Africans). This stage of *acute infestation*, during which some die and nearly all are seriously ill, is only dangerous for a comparatively short period, and is definitely on the wane within a year. This is followed by what we have called the stage of *semi-immune infestation*, a period during which there is no danger to life, but in which there are wide fluctuations in the parasite count. After the fifth year there is a constant decline in the number of parasites seen in the peripheral blood, and after the age of 10 or 12 the stage of *immune infestation*, as described by CHRISTOPHERS (1924), is fully established.

The maximum spleen size occurs in the stage of acute infestation, and thereafter the spleen progressively declines up to adult life. The average enlarged spleen up to the age of 10 is 1·9, and the Spleen Rate 85 per cent., but the maximum frequency of enlarged spleens is in the stage of semi-immune infestation, that is in the age-group 1 to 5.

3. KHONDS.

The Khonds are a people which has been settled in the Jeypore Agency Tracts of Orissa for hundreds, and perhaps for thousands, of years; they are largely Pre-Dravidian in origin, and the Pre-Dravidian physical type is prominent among them. Between 20th March and 7th April, 1935, blood films were taken from 98 persons, and the spleens of 230 children examined, so far as possible from pure Khonds. The numbers examined are so small that the results would not by themselves be very convincing; but they are so similar to those recorded by CHRISTOPHERS (1924) in Singhbhum and, in more general outline, by PERRY (1913) in the Jeypore Hill Tracts, that there can be little doubt of their substantial accuracy.

TABLE VI.

SPLEEN EXAMINATIONS IN KHONDS AND DIGO.

Age.	Khonds.			Digo.		
	Palpable.		Average Enlargement.	Palpable.		Average Enlargement.
	Positive.	Negative.		Positive.	Negative.	
0–1	5	1	3·6	16	2	3·6
1	15		2·8	8		2·8
2	33		2·4	6		1·8
3	13		2·4	8	1	2·1
4	12		1·9	9		2·0
5	7		1·1	7	1	1·6
6	5		2·0	9		1·3
7	12	1	1·8	3	4	1·7
8	14		1·8	9	3	1·7
9	4	1	1·3	2	1	1·0
10	8	2	1·5	6	4	1·8
Percentage { Age 1–5 ... 100 Age 6–10 ... 92				Percentage { Age 1–5 ... 94 Age 6–10 ... 77		

The Spleen Rate in these Khond children was 97 per cent. and the average enlarged spleen 2·1; none of these apparently receive any treatment. In a group of higher caste children (these being more Aryan in type) who were also examined at the same place, there was not, however, any significant difference in the average enlarged spleen (as determined by this method), nor in the distribution of the several spleen sizes, although the Spleen Rate was 8 per cent. lower. This larger number of negatives was probably explained by the presence of a few who had been treated with quinine.

The spleen and parasite findings are shown in Tables VI and VII, and the parasite counts in Fig. 5. They are surprisingly similar to the Digo series with which they are there compared. It can safely be assumed that the course of infections in adult life would be equally similar.

The gametocyte rate in children aged 1 to 5 was 24·7 per cent., as compared with a mean rate of 34·5 per cent. in the Wadigo ; but as these films were taken at the period of least anopheline prevalence, SENIOR WHITE (1935), the gameto-cyte rate should perhaps be compared with the corresponding Digo rate of 26·8 per cent.

The chief fact which emerges from the comparison of these two " immune " communities is that, in spite of wide differences in race and physical surroundings,

TABLE VII.

BLOOD EXAMINATIONS IN KHONDS AND DIGO.

Age.	Khonds.			Digo.	
	Parasites.		Parasite Count.	Parasite Count.	Parasite Rate.
	Positive.	Negative.			
0–1	2		9300	7875	87·5
1	11		5168	7176	100·0
2	12	1	4998	4732	100·0
3	18		2125	2702	100·0
4	12		1294	1796	97·7
5	10		850	828	97·4
6	8		1204	466	88·5
7	3		1260	791	79·0
8	6		770	427	74·1
9–25	9	6	308	237	58·0

Species at Age 1–5 :
    P. falciparum ... ... 87·9 per cent.   84·7 per cent.
    P. malariae ... ... 27·6 ,,   41·7 ,,
    P. vivax ... ... 29·3 ,,   20·1 ,,

communities in India and Africa have yielded the same endemic data when compared by the same methods of examination.

4. MIXED BANTU COMMUNITIES.

In the course of the discussion of the data already given, reference will be made to two other series of examinations which we have made, WILSON (1936a), at Tanga and Amani. The former is a small coastal town in Tanganyika with a

very mixed population of Bantu, consisting mostly of individuals with a fair amount of immunity to malaria. As a fairly effective anopheline control has been established in Tanga, the majority of infections are acquired outside it. The results of examinations are summarized in Table VIII, and they are typical of the findings in East African towns in endemic areas.

TABLE VIII.

SPLEEN AND PARASITE RATES AT TANGA.

Age Group.	Spleens.		Parasites.		
	Number Examined.	Per cent. Palpable.	Number Examined.	Per cent. Positive.	Average Count.
0–1	82	20·7	810	16·8	3455
1–5	198	63·0	236	72·5	1843
6–10	240	63·4	390	69·5	424
11–20	182	39·0	539	47·0	436
Over 20	687	28·4	883	25·8	167

Amani is a small station in the hills, and the infections shown by the people examined are acquired in visits to tne vailey at their foot. The data summarized are not, therefore, representative of malaria endemic at Amani; but, even if the numbers examined are too small for any wider conclusions to be drawn from them, the results are sufficiently interesting for the purpose of the present discussion. This Bantu community shows a range of spleen-parasite relationships entirely different from those of the other Bantu communities studied, and intermediate between these and the findings of SCHÜFFNER in Malays.

TABLE IX.

SPLEEN AND PARASITE RATES AT AMANI.

Age Group.	Spleens.		Parasites.	
	Number Examined.	Per cent. Palpable.	Number Examined.	Per cent. Positive.
0–1	14	43	10	70
1–5	29	76	20	75
6–10	18	89	17	76
11–20	77	74	52	50
Over 20	176	45	153	28

IV.—INFECTION AND MORBIDITY IN THESE SERIES.

It is extremely difficult, and often impossible, to compare the infected anopheline infestations recorded by different observers (when they are recorded) ; but as we believe the intensity of the infecting and immunizing agents to be of paramount importance in determining the relationship of the spleen and parasite rates, some attempt is made to compare the chances of infection in the villages under consideration. It is very little easier to make a satisfactory comparison of the resulting immune status in the communities themselves, but a broad division can be made into those which are immune and those which are sub-immune.

SWELLENGREBEL and his co-workers (1931) state that anopheline infestation was much greater in their South African villages than in those in the Dutch East Indies, while in the latter the chance of infection was put at four times a year. With regard to immunity, they repeatedly refer to the freedom from fever of the Bantu, even as children ; but SCHÜFFNER (1919) states that there was an excess mortality of 20 per cent. in the malarious, as compared with the healthy, areas of the Dutch East Indies.

With regard to the Mysore series, SWEET and RAO (1931) found less than 1 per 1,000 infected in some 14,000 dissections of the only vectors in the area ; the chance of infection does not appear to have been more than about once a year. From the data given by MACDONALD and MAJID (1931), the chance of infection in their series should have been even less than once a year. SWEET (1933) records a considerable morbidity and mortality from fever in Mysore (a morbidity which was reduced by anopheline control measures) ; and MACDONALD and MAJID remark that although there was evidence of lessened susceptibility in older children, yet even in these there was a considerable increase in the frequency and intensity of infections during the malaria season.

The contrast between such communities as these and the Khonds whom we examined is best shown by the fact that if plains-people, for example Punjabis, reside in these Agency Tracts, they suffer from constant incapacitating attacks of malaria ; while the Khonds themselves were obviously healthy and fertile, although no vital statistics of any value are available to confirm this opinion. Anopheles of carrier species are present in their country in enormous numbers, and re-infections must be frequent.

The mean infected anopheline infestation in the Digo area was 0·55 per house and we estimate the frequency of infection at about thirty times a year, varying from three times a week to once a month ; yet in spite of this degree of infection in the locality, we were unable to find any morbidity attributable to malaria after the first two or three years of life. The only reliable vital statistics that have so far been obtained in East Africa have been reported by PHILIP (1933), and apply to another, similarly hyperendemic, area of the Digo country. The infantile mortality attributed to malaria in this area is 11·8 per 1,000, which may be compared with the case mortality in the Ceylon epidemic of 40 per 1,000.

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In the Tanga area the liability to infection is much less than in the Digo villages, probably not more than half ; at Amani it is dependent on the frequency of the individuals'·visits to the lower levels ; and in Masailand it depends on the movements of the " village " in search of water for the cattle.   We are unable to make any estimate of the morbidity suffered by these communities on account of malaria, but it is well known, both by themselves and others, that the Masai are at all ages very susceptible to attacks of malaria, both in its milder and most severe forms.

V.—Theories of Immunity in Malaria.

In experimental infections of monkeys it has been found (see especially TALIAFERRO, 1932) that immunity in malaria depends on the reticulo-endothelial system.   Forty-eight hours after infection there is marked concentration of parasites in spleen and liver ; there is also increased activity of the macrophages and proliferation in the spleen follicles with consequent enlargement.   In the later stages of the infection this macrophage activity is maintained and there is in addition a *quickened* cellular response to reinfection.   Although parasites may not at this stage be apparent, the blood is still infective to non-immune animals and if, in such a premunized monkey, the spleen be removed, an acute relapse of malaria follows, MALAMO (1934), KNOWLES and DAS GUPTA (1934).

These results are in line with the observations on birds of various workers, see Summary by TALIAFERRO (1930), the immune reaction in either case depending on an increased cellular reactivity.   There is no reason to doubt that the same mechanism operates in man, and that splenic enlargement is a reflection of this process.

This is not, however, the complete story.   It has long been recognised that there are two types of splenic enlargement, depending on two stages of the pathological process.   The soft large spleen of the non-immune is not immediately comparable with the harder and relatively smaller spleen of immune individuals. The former depends on acute congestion and the early cell proliferation already referred to as occurring in monkeys, while the latter represents the reaction of established immunity.   The appearance of this latter type of spleen is characteristic (TALIAFERRO, 1932), and it is the result of a specific response to the antigen which evokes it.

But the response to certain antigens, and we suggest that some part of plasmodial substance is one of these, consists of two separable reactions, namely, the immune reaction itself and an allergic reaction.   The allergic reaction occurs in various organs and consists of cell proliferation with hyperaemia and oedema, which are strikingly increased in intensity and rapidity compared with the normal tissue.   The reaction occurs in previously infected animals only, and differs from a first infection in this striking rapidity and severity.   A typical

example of it is the allergy of tuberculosis, in which an inflammatory reaction may go on to cell-death ; and this may occur both in the skin and in internal organs.

In some experiments on animals infected with *Pasteurella aviseptica* and pneumococci, RICH, JENNINGS and DOWNING (1933) have shown that when the hypersensitivity (of animals previously rendered allergic to these organisms) is completely abolished by the intravenous injection of large doses of the killed organisms, the immunity to subsequent virulent infections remains intact.

When an individual is exposed to repeated frequent reinfection, as occurs in a hyperendemic malarious area, we postulate that such a process of desensitization is actually taking place, while immunity is at the same time increasing. When infections are less frequent *some* immunity may be developed, but the concurrent development of allergy is not prevented. CUMMINS (1934), speaking of the tuberculin type of hypersensitivity, remarks : " It would seem indeed that one of the principal functions of ' acquired immunity ' is to protect the infected individual from the effects of hypersensitivity."

One of the best known and most easily demonstrated allergic reactions is the intradermal tuberculin reaction. It has been shown that in Bantu there is a quantitative relation between the degree of reaction and the liability to tuberculosis (*Tuberculosis in South African Natives*, 1932). The intradermal reaction with digested malaria parasites described by SINTON and MULLIGAN (1932) appears to show that a similar hypersensitivity to infection occurs in monkey malaria. Positive reactions were obtained in both acute and chronic infections in non-immune monkeys, and the mechanism of this reaction is evidently closely allied to that of the tuberculin and similar cutaneous reactions.

VI.—THE SPLEEN RATE.

The foregoing exposition of some of the more recent findings in avian and simian malaria is essential to the understanding of the explanation which we wish to put forward of the contrasts and similarities in the malarial data regarding the communities here discussed. The recognition of the part which allergy plays in tuberculosis has done much for the understanding of that disease and it has seemed to us that it might play an equally useful part in the understanding of malaria.

The intensification and acceleration of response to infection characteristic of allergy is present in the behaviour of the reticulo-endothelium of infected monkeys, and is also shown in the greater liability to persistence of splenic enlargement during the whole life of those who are not fully immune but still hypersensitive. The allergic state may also provide some part of the explanation of the chronic relapsing malaria, and haemoglobinuria, to which such persons are liable.

Figs. 1 to 4 show the spleen and parasite rates in seven of the communities cited (the only examples available to us in which sufficient and comparable data are given). It will be seen in both series of Fig. 1 and in the Malay series of Fig. 2, that the spleen rate is greater, more or less, than the parasite rate, and shows little change with age. In Fig. 3, the spleen rate shows a well-marked decrease with age, but is still greater than the parasite rate. In the South African series of Fig. 2 and the Tanga series of Fig. 4, there is an even greater decline with age in the spleen rate, and the two rates closely approximate. In the Digo series of Fig. 4, the spleen rate shows a greater decline with age, and is always less than the parasite rate. Of these series we would regard only the last as representing the fully immune status, while the remainder represent successive levels in its development. In the fully immune community the adult spleen rate is formed solely of immune spleens, that is spleens which show no variation in size (we have ourselves observed this over a period of 2 years), most of them being so small as to be impalpable or only just palpable, and a few which are larger depending in all probability on past history or individual resistance; but not, it should be noted, on the presence of an admixture of the large spleens of the acute or the hypersensitive type. In such communities immunity is no longer qualified by the persistence of hypersensitivity.

The two series (South African and Tanga) in which the spleen and parasite rates are crossing one another, closely approximate to the fully immune.

The other four series, Indian, Masai, Malay and Amani, represent varying degrees of what may be described as sub-immune communities, of which the most typical example is the Indian series (Mysore). Although in this series the chances of infection were low (see above), the Spleen Rate in children is as high as that at Tanga, and higher in adults than either this or the Digo series. We interpret this adult spleen rate as being a combination of " immune " spleens (in some of those who had not recently been infected) and a probably larger number of spleens which were in a state of allergic reaction to more or less recent reinfection. It is of great interest to us that such a sub-immune condition should be found in the Bantu series at Amani, that is in a locality where infections are of sufficient infrequency to produce it. In sub-immune communities the immune process has not gone on to the final stage of desensitization.

VII.—THE PARASITE RATE.

It will be observed from Figs. 1 to 4 that the infantile parasite rates may* be arranged in an ascending order, which corresponds in an inverse ratio with the spleen rate. It is equally evident that this is only an approximation, but it suffices to distinguish the immune and sub-immune communities. In the latter group the reinfections which occur are spaced at such long intervals that they

*With the exception of the Malay series : see note on Fig. 2.

INDIAN AND MASAI SERIES

SPLEEN RATE

SPLEEN RATE

PARASITE RATES

PERCENT

AGE 5 10 15 20 25 30 35

INDIAN ———— MASAI ————
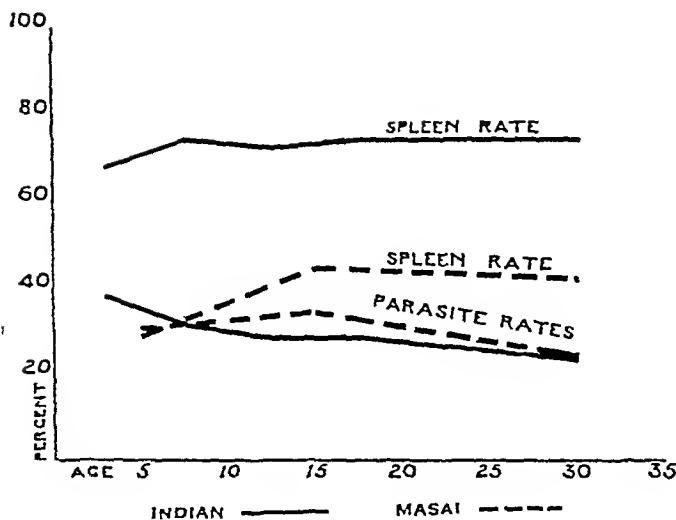
FIG. 1.

MALAYAN AND SOUTH AFRICAN SERIES

SPLEEN RATE

SPLEEN RATE

PARASITE RATE

PARASITE RATE

PERCENT

AGE 5 10 15 20 25 30 35 40

MALAYAN ———— SOUTH AFRICAN ————
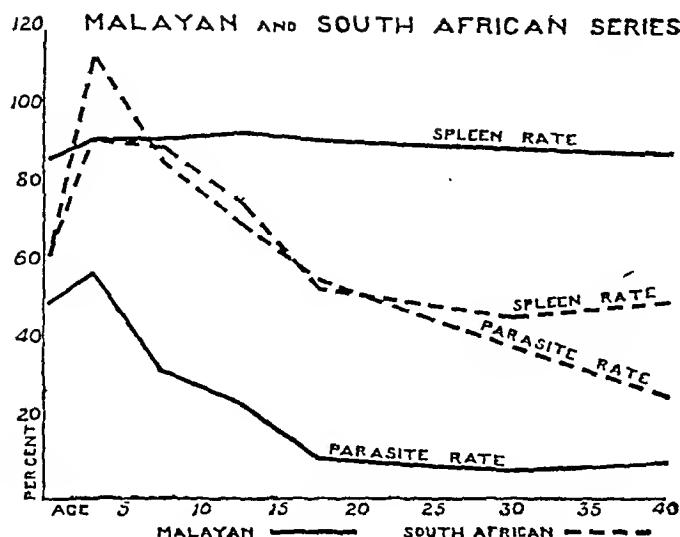
FIG. 2.

The rates shown in Fig. 2 are, in comparison with the other figures, too high; as mixed infections are counted more than once.

AMANI SERIES

SPLEEN RATE

PARASITE RATE

PERCENT

AGE 5 10 15 20 25 30 35 40

FIG. 3.

DIGO AND TANGA SERIES

DIGO

TANGA

PARASITE RATE

SPLEEN RATE

PARASITE RATE

SPLEEN RATE

PERCENT

AGE 5 10 15 20 25 30 35 40
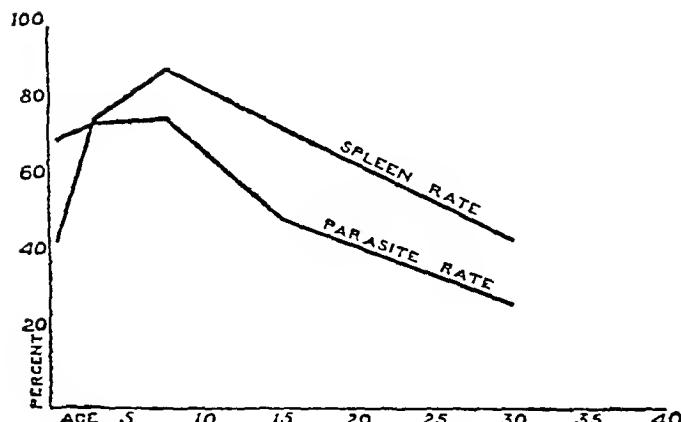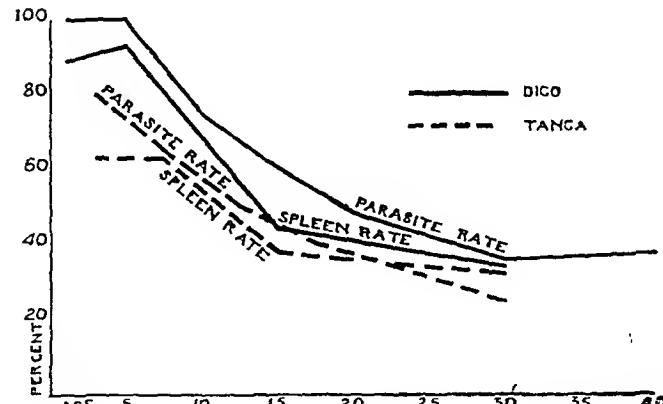
FIG. 4.

may be treated as separate infections, and the parasite rate can be explained in terms of chance distribution. To this extent we follow the theory of discrete infections advanced by MACDONALD (1930).

But when the infantile parasite rate is high (at least over 70 per cent.), the infected anopheline infestation is very much greater, and reinfections are too frequent for recovery to take place in the intervals between them. In such communities the parasite rate at any age is, therefore, the resultant of the infections distributed and the degree of immunity already acquired (in Tanga, for example, although the immunity in adults was rather lower than in the Digo, the chances of infection were very much lower, and the parasite rate is accordingly 12 per cent. lower). In this type of community the overlapping infection theory of CHRISTOPHERS (1927) seems to be the true explanation of the parasite rate, and this explanation applies to most hyperendemic African villages, as described by SWELLENGREBEL and his co-workers, to the Khond villages and to those described by CHRISTOPHERS (1924) in India.

VIII.—THE INFLUENCE OF RACE ON THE ENDEMIC STATUS OF A COMMUNITY.

The succession of intermediates shown (particularly by the Bantu) in these series appears to prove that there can be no clear-cut racial distinctions in the immune reaction to malaria. It may be that the greater contrasts found in a country are associated with race ; but we believe that the reasons for such contrasts in the malarial status are primarily historical or social and not a function of some inherited difference in immunity. The Masai for example live on their arid steppes for the sake of their cattle, and not because of their fear of malaria.

We have been quite unable to distinguish first infections either in African infants from endemic areas, in adult Africans from non-endemic areas, in Indians or in Europeans, either by their clinical severity, by the parasite count, or by the morphology of the parasites seen. Such races as the Malay or the Indo-Aryan may be incapable of developing a complete immunity ; we are of opinion, from our study of the literature, that there is insufficient evidence to give an answer to this question. But that Bantu or Pre-Dravidian communities owe their immunity to an innate tolerance we hold to be untrue. Our own observations in East Africa lead us to the conclusion that frequent reinfections are necessary for the development and maintenance of complete immunity under natural conditions, and this should apply in any country. The immune status of a community is then dependent on the infected anopheline infestation of the locality, and the implication of our argument is that if immunity be obtainable in one race it is obtainable in another. Whether, however, its achievement can be so modified that it ceases to be a menace to life and health remains a suggestive speculation.

IX.—THE MEASUREMENT OF ENDEMICITY AND THE IMMUNE STATUS.

The indices which have been so widely used in the estimation of the endemicity of malaria have proved of great value in comparing the degree of malaria present in different communities ; this has been because in each country the majority of the population examined has been living under similar conditions, and the standard applicable to this majority has been applied. But it is evident from the various examples which we have quoted that, when conditions are not similar, the Spleen Rate (in children) is no reliable criterion either of the malariousness of the locality or of the immune status of the community. It represents rather the degree to which that particular age-group is subject to attacks of malaria. The best example of the potential fallacy of the Spleen Rate is shown in Fig. 2.

The Endemic Index (or parasite rate in children) is evidently a more valuable guide to the incidence of malaria in a locality and in the community as a whole. By a comparison of Figs. 1 to 4, it can be seen that the relationship of the spleen and parasite rates, and their probable course throughout life, can approximately be estimated from the parasite rate in the earliest age-group. In other words the probable degree of immunity that is present in the community can be estimated. Moreover, since this is a parasite rate in non-immune or only slightly immune, children, it will be a very fair measure of the chance of infection in the locality (provided no measures of personal protection are in use). The most accurate measure of this kind is the annual rate of infection in early infancy (STRICKLAND and SEN GUPTA, 1936), but this is rarely obtainable and the rate in children up to the age of 4 or 5 would be a very good substitute for it.

If older children, up to say the age of 10, are included in the Endemic Index, its accuracy is greatly diminished ; in fact, the fall, or lack of fall, between the age-groups 1 to 5 and 6 to 10, gives some notion of the immunity present in the community as a whole. If the ratio between the child and adult parasite rates be taken, an index is obtained which is still only a rough indication of the immune status of the community, and the same qualification must be made of any ratio between the spleen rates at different ages.

From the data given in this paper, it would in fact seem that the only way of comparing the immune status of communities in which the general epidemiological surroundings are dissimilar is by a study of the spleen and parasite rates at least up to adult life.

There remains, however, one much more sensitive index of immunity, namely, the variation in the average parasite count in infected persons. It is unfortunate that, with the notable exception of workers in the Malaria Survey of India, it has been so little used in epidemiological studies of malaria. On this account we have been unable to use parasite counts in the present discussion ; but we should like in conclusion to make some reference to them, in the hope that other workers may be incited to make use of this method and so to provide

data of sufficient precision to lift epidemiological comparisons from the level of intelligent guesswork.

In Fig. 5, we have plotted the results of parasite counts in the Digo and Tanga series, of CHRISTOPHERS' and our own series in Orissa, and of the Masai series. Of these only the last is of the non-immune type, and the numbers of
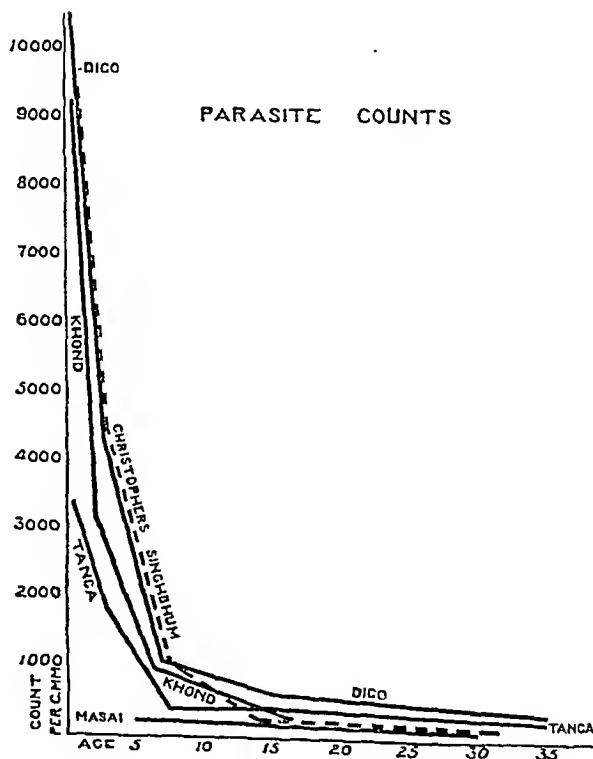
FIG. 5.

positives were unfortunately small. There is a remarkable correspondence between these curves of immune communities in two continents; whereas the sub-immune community shows no appreciable change with age, there is an enormous difference between the child and adult counts in the remainder.

We suggest that it is by the use of parasite counts that the varying findings in different countries and races may be correlated, and a true estimate of the immune status be made.

SUMMARY.

1. The results of various studies by other workers on the spleen and parasite rates in Indians, Malays and Bantu are summarized.

2. The results of our own observations on Khonds in India, and on Masai and Bantu in East Africa are also summarized and compared with the foregoing and with one another.

3. It is pointed out that wide differences may be found in the malarial status of communities in the same country and, in the case of the Bantu, of the same race ; while close similarities occur in different countries.

4. The results of experimental infections in birds and monkeys are cited and interpreted in terms of the two states of allergy and immunity. The probable significance of these is illustrated by reference to the role of the two states in tuberculosis.

5. It is suggested that the observed contrasts in the spleen and parasite rates in diverse communities may also be explained in terms of immunity and hypersensitivity.

6. It is concluded that the immune status is dependent not on race but on the frequency of infection ; and that the most accurate index of endemicity is the frequency of infections in the non-immune, that is the rate in early infancy.

7. While a consideration of the Spleen Rate and Endemic Index may be of value in forming an estimate of endemicity and immunity when similar communities are being compared, these indices may be misleading when the epidemiological surroundings are dissimilar.

8. A consideration of the variation with age in the spleen and parasite rates throws more light on endemicity and immunity, and on such variation a rough classification may be made into *immune* and *sub-immune* communities.

9. But it is suggested that by far the most sensitive index at present available for field studies is the average parasite count, and its variation with age.

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MALARIA IN TRINIDAD. LOW TIDE LEVEL CULVERT SYSTEM IN COASTAL DRAINAGE.

BY

ERIC J. DE VERTEUIL, M.D., B.S. (LOND.), D.P.H. (LOND.)*

Senior Medical Officer of Health, Trinidad,

AND

T. SPENCE, A.M.INST.C.E.,

Water and Sanitary Engineer, Trinidad.

PREVIOUS HISTORY OF MALARIA IN BRIEF.

Malaria in the West Indies has had a long past but a short history, and this general position is well reflected in the history of malaria in Trinidad where the most recent scientific knowledge in connection with the prevention of malaria is now being applied for the purpose of its complete eradication in selected coastal areas as well as for its general control over the rest of the island.

We know from documents and plans collected by the Trinidad Historical Society that, between the years A.D. 1600 and 1700, during the early Spanish occupation of Trinidad, our present capital city, Port of Spain, was represented merely by the tiny Indian coastal settlement of Mucurapo, whilst the two main Indian missions were situated far inland at Arima and Princes Town about 16 and 8 miles from the coast respectively.

Later on, during the 18th century, the Spaniards also made their capital town St. Joseph 6 miles inland, while it has required about one and a half centuries of gradual development for Port of Spain (population now about 72,000) to grow out of the low-lying mangrove swamps of the Caroni Delta (now known to be the home of *Anopheles tarsimaculatus*) and of the very attractive surrounding hills and valleys.

During this period, and up to about 40 or 50 years ago, malaria (then usually called " remittent," " bilious remittent " or " typho-malarial fever "), yellow fever and typhoid fever formed together one group of diseases whose ravages

among the population of the small West Indian Islands and especially of the small white section, has often been described in text books and epidemiological papers. Trinidad shared freely in this annual wholesale destruction of human life at a time when any possible escape from such diseases had not yet come within the scope of scientific thought and action.

Yellow fever was the first of this triad to give way to the application of science in man's progressive march into these unhealthy tropical regions ; and in 1914 the last sporadic cases of yellow fever occurred in Trinidad.

The high incidence of typhoid fever with its attendant mortality has also yielded to the persistent scientific efforts of the Public Health Department and has shown a gradual and considerable reduction ; and it is confidently anticipated that a permanent low level will soon be reached with the early completion of the Central Water Supply Scheme at a cost of approximately £1,000,000.

Malaria, however, still remains with us, though its incidence and mortality have shown a considerable reduction in the towns and villages and in certain industrial areas where the usual anti-malarial measures have been applied, including drainage, screening, and effective early medical assistance and also improvement in housing and living conditions generally.

RELATIVE EFFICIENCY OF VARIOUS FACTORS IN THE CONTROL OF MALARIA AND THE POSSIBILITY OF HASTENING SUCH CONTROL.

JAMES (1929) in England and BARBER (1929) in the U.S.A., have both pointed out the advisability of studying the history of malaria in a country in order to estimate correctly the influence of the various factors which may be concerned in any decrease of malaria.

It now seems opportune to attempt to evaluate briefly the principal factors which appear to have been responsible for this decrease of malaria in Trinidad in the past and at the same time to consider the possibility of (a) hastening its diminution in the future and (b) of procuring its total eradication in some of the smaller West Indian Islands—by the application of such measures as form the subject of this paper.

Agricultural Development.

Previous to 1905, in the absence of precise scientific knowledge, there was, as might be expected, no fixed anti-malarial policy outlined in connection with malaria control, but the gradual social and industrial development of Port of Spain and the larger towns along the coast brought naturally in its train a slow but steady decrease in the extent of the adjoining brackish water swamps, in which our principal malaria carrier, *A. tarsimaculatus*, is now known to abound. The marked success of these measures naturally proved to be a factor of the greatest importance in the future selection and development of village sites along

the coast. Whilst these coastal improvements took place, the healthier inland towns and villages also shared in this gradual economic development of the island, and large agricultural enterprises and schemes of drainage and improved sanitation had already begun to evolve. The other factors which we now usually consider as being of definite anti-malarial importance, such as anti-larval measures, effective medical assistance, and good living and housing conditions do not appear to have played any influential part at this stage in the control of malaria.

Up to about 1905, therefore, the natural economic development of Trinidad was the sole factor of importance in the control of malaria, and this development occurred almost purely as the result of intensive agricultural efforts (principally in connection with cocoa and sugar-cane) in a land with wonderful climatic, rainfall and soil advantages.

We particularly wish to stress the agricultural nature of this development and progress in connection with malaria control, because we feel that most of the smaller West Indian Islands have not yet advanced beyond this stage, a stage which may be considerably shortened if some method of hastening its development such as is now suggested is proffered to them.

Anti-Malarial Drainage.

With the construction of the Panama Canal in 1905 and the development of new methods of larval control by GORGAS and his associates, the medical authorities in Trinidad were keenly alive to the immense possibilities not only of improved malaria control but also of general sanitary improvements ; and the sterling work carried out in this direction between 1910 and 1930 by J. R. DICKSON and the late C. F. LASALLE is fully recognized in Trinidad and throughout the West Indies. The drainage work consisted, briefly speaking, of general drainage measures (permanent or otherwise) in close proximity to populated areas.

During the whole of this time, the agricultural development of the island continued to play a leading part in the diminution and control of malaria, and in this connection the Colony owes a deep debt of gratitude to the large sugar estates whose extensive drainage schemes stand out even to-day as models for all others.

The Quinine Factor.

The free use of quinine on the sugar and cocoa estates and the gradually improved and more generally accessible Government medical assistance which had become available owing to the marked improvement in road and travelling facilities were also very important factors in the fight against malaria.

Housing and Living Conditions.

In 1918, with the introduction of the new Public Health Laws which necessitated control of the construction of all new houses and of all sanitary measures (including drainage) by the various local health authorities which were appointed at this time, marked strides were made in the development and amelioration of the housing and living conditions of the population generally ; but this became especially evident in the rural districts which until then lacked the necessary and requisite amount of guidance and encouragement.

The Nutrition Factor.

As nutrition is now recognized to be such an important factor in the control and prevention of all diseases including malaria, it becomes necessary to point out briefly the improvements which have been made during the past 15 to 20 years in the food supply in Trinidad.

Previous to 1918, and especially during the years of the Great War, malnutrition was clearly manifest in the prevalence of anaemia, scurvy, poor musculature, skin conditions, bad teeth, xerosis, night blindness and chronic fatigue ; and this was particularly marked among the East Indian section of the community. The diet of both the upper and lower classes, including all the different races, may be considered as being then generally grossly inadequate, so far as our present standard of requirement goes. Fresh milk, fresh green vegetables, eggs, fresh butter, meat and fresh fish were generally expensive and scarce, and the same applied to fresh fruit in many localities.

To-day, all these articles of food are easily and cheaply procurable in the larger towns and villages and a fair consumption of fresh fruit and fresh green vegetables is more or less general throughout the island and there is also a definite tendency to an increased consumption of fresh milk and fresh butter. The East Indian race, however, forms a notable exception to the above, and special economic, agricultural and educational efforts are now being made to improve their housing and living conditions. Improvement of nutrition among the population generally is now clearly evident as compared to pre-war years, and the improvement of the standard of physique among the school-children is especially manifest.

We may safely conclude, therefore, that the improvement in the housing and living conditions of the population has had a very definite bearing on the diminution of malaria.

The Oil Industry.

Last, but by no means least, as a factor of the greatest importance in the diminution of malaria must be mentioned the gradual development of the oil industry in the southern portion of the island since about 1918. The principal oil companies have from the very outset taken the keenest interest and

kept abreast of the most recent developments in the prevention and control of malaria. It is due largely to their thorough and progressive anti-malarial policy that screening of bungalows and improvements in medical assistance, as well as in the housing and living conditions of the labouring class, were rapidly established not only on the oilfields and in their refining centres but also in connection with the other industries of the south of Trinidad. The early screening of government officers' quarters was also a direct offshoot of this advanced anti-malarial policy.

Apart, however, from providing such local anti-malarial benefit, the oil industry continues to be the principal financial mainstay of the Colony and has acted as its " guardian angel " and protector at a time when its agricultural resources were seriously endangered and malaria threatened to raise its avenging head once more.

It should be borne in mind that all these factors which have been operating at various times and in different parts of the island are clearly interdependent and mutually co-operative in nature and it is not possible to evaluate correctly their relative efficiency ; but they are all definitely and closely dependent on the continued agricultural development of the Colony.

The Malarial Survey.

Until 1930, therefore, our anti-malarial policy was based generally on the various measures mentioned above. By this time, however, it had become clear that any further progress in anti-malarial work depended on a careful survey of local conditions. A malaria survey (including anopheline and topographical surveys) was, therefore, arranged for, and one of us (E. de V.) was placed in charge.

As a result of this survey, it is now recognized that of thirteen known species of anopheles, two only play an important part as malaria carriers in the transmission and spread of malaria : (1) *A. cruzi*, and (2) *A. tarsimaculatus*. Intensive anti-malarial control work in connection with these two anopheles seems, therefore, to be clearly indicated.

Anopheles cruzi (A. bellator).

This anopheles is essentially a forest mosquito and is found in large numbers in the hilly inland portions of the island where the rainfall is sufficient to maintain a prolific growth of water-holding bromeliads (locally known as " wild pines "). These parasitic plants grow plentifully on the trunk and branches of the forest trees and hold sufficient water at the base of their leaves to permit of extensive anopheles breeding during the rainy season. Under such conditions, the anopheles bite freely during the day in the shade of the forest but are especially active towards dusk and for an hour or two after.

Ordinarily, therefore, as forest conditions obviously preclude a large human population, this mosquito could not be of any importance in the spread of malaria. Over large tracts of the island, however, such forests have been cut down and replaced by cocoa estates on which the "mother" or shade tree of the cocoa, the "immortelle," has been extensively planted.

It is unfortunate that under such conditions these immortelle trees harbour a phenomenally large number of bromeliads (in some instances probably 500 to 1,000 per tree) with the result that the villages which have usually grown around such estates become infested with the anopheles from neighbouring immortelles, and in this way a harmless forest mosquito has become a very dangerous malaria carrier which is probably responsible for about 20 per cent. of the mortality from malaria.

These tiny mosquitoes (which are about half the size of *A. tarsimaculatus*) swarm in large numbers for a period of about 6 to 8 weeks from June to August, and begin to bite freely at about dusk and continue to do so for about one to two hours after. They are commonly caught in the yards, sheds, stables, out-houses and galleries, but also enter houses, although to a much more limited extent. This anopheles should, therefore, be classed as a notable exception to the rule that all important malaria carriers are essentially house-haunters. In this instance, the transmission of malaria appears to take place almost wholly outside of the houses.

The control of *A. cruzi* is obviously a very difficult question which will require further consideration.

Anopheles tarsimaculatus.

A. tarsimaculatus (recognized as one of the most efficient malaria carriers in South America) is essentially a brackish water mosquito which is able to breed permanently throughout the year in all the suitable coastal swamps of the smaller West Indian Islands.

In Trinidad it has been estimated that about 70 per cent. of the mortality from malaria during 1931 to 1933 was caused by *A. tarsimaculatus* and that the cases occurred within 4 miles of brackish water breeding swamps (*Annual Report of the Surgeon-General, 1933*, 1934); whilst EARLE (1936) states that in Grenada and St. Lucia this mosquito was found everywhere associated with malaria and that it is obviously the cause of most or all of the malaria in these two islands. There is also every likelihood that it is equally responsible for the malaria in the other islands as well.

In Trinidad, *A. tarsimaculatus* breeds out in enormous numbers in the early part of the rainy season in coastal swamps and river mouths (even in water having a salinity up to 70 per cent. of equatorial sea water) and then migrates inland for a distance of at least 3 miles into less malarious areas where it breeds freely (though only for two or three generations) in rice fields, stagnant

drains, etc. In this way, it is able to spread malaria at this season over the greater part of the island.

Its continuous existence in any locality during the dry season is, however, dependent on the presence of permanent brackish water swamps. Many of these brackish water swamps dry up completely during the dry season, while others become too saline to permit of anopheles breeding. There are, unfortunately, however, in Trinidad many large swamps and river mouths which maintain a sufficient salinity to allow of anopheles breeding throughout the year, and these constitute their permanent reservoirs.

We consider that a severe drought of about three months (which is not uncommon in the West Indies) is sufficient, in the absence of suitable breeding places, to wipe out all the *A. tarsimaculatus* in a locality even under good conditions of shelter for the adults. There can be little doubt that the recent disappearance of malaria from the exposed and wind-swept island of Barbados after severe epidemic conditions lasting about 3 years from 1927 to 1930 was due to such a drought, a fortunate event, as there then existed only three coastal swamps which were capable of mass breeding of anopheles in the rainy season, and these, during the prolonged dry spell which occurred must have become too saline to permit of the continuance of such breeding. The possibility, however, of the re-introduction of this mosquito in the near future from the neighbouring islands or British Guiana in schooners or small craft cannot be overlooked.

We conclude, therefore, that *A. tarsimaculatus* cannot continue to maintain an existence for any prolonged period of time on a small West Indian Island which does not provide the requisite number, and the right kind, of coastal swamps and river mouths in addition to fresh water inland pools.

It appears likely that, in addition to Barbados, St. Vincent and probably some other smaller islands may prove to be of this nature.

THE LOW TIDE LEVEL CULVERT.

As malaria, however, is extremely prevalent over most of the other islands, our past and recent experience in Trinidad, and a limited knowledge of conditions in some of the other islands, as well as the recent Barbados experience, all lead us to believe that any measure, which, at a reasonable and economical expenditure, is capable of rapidly and permanently eliminating anopheles breeding in such swamps and river mouths and of so preventing their spread into more healthy areas, should prove to be a welcome addition to our armamentarium in the fight against malaria, not only in the West Indian Islands, but also in other parts of the world where brackish-water-breeding anopheles are a menace to public health.

We are of opinion that such swamp clearance would be strictly comparable to the slum clearance which is now being undertaken in all our towns, not only for the benefit of actual slum dwellers, but also to safeguard from infectious

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diseases a population living under good conditions in close proximity to a population living under slum conditions and in a state of unstable equilibrium.

It seems to be just as clearly indicated as a remedy that all dangerous malarious swamps should be cleared from our midst as rapidly as we are now ridding ourselves of the slummy congested areas of our towns.

It is with this end in view, therefore, that we append herewith some observations on the Low Tide Level Culvert System which was started in Trinidad and Tobago 3 years ago and which continues to give satisfactory results.

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APPENDIX.

SOME OBSERVATIONS ON THE LOW TIDE LEVEL CULVERT SYSTEM FOR DRAINAGE OF COASTAL SWAMPS AND RIVER MOUTHS.

General.

1. In Trinidad and Tobago there are numerous swampy areas adjoining the sea.   Their natural characteristics vary to a large extent depending upon their size, depth of water in wet and dry seasons, location, whether influenced by the heavy seas of the Atlantic or the comparatively calm seas of the Gulf of Paria, their immunity from or their subjection to flooding at high tides and the height and length of the sand-bank, rainfall and extent of catchment area, etc.

Tidal Range.

2. There is a mean tide range between high and low tide of $3\frac{1}{2}$ feet.   In May and October, however, the range is about 5 feet and at these two periods, especially if helped by wind and wave action, the swamps are flooded to the greatest extent.

3. For many years, the Trinidad Government has been spending annually a considerable sum of money in attempting to keep clear the mouths of tidal rivers, streams and drains.   This was done by manual labour with little or no success as far as the elimination of anopheles breeding was concerned.   Their efforts were usually nullified, even the same day, by wind and wave action again " banking up " sand and gravel.   Considerable intervals—in some cases months—took place between clearances.

Types of Swampy Areas.

4. These fall roughly into four categories :

(*a*) Swamps in or on the outskirts of important towns, where, building site value being high, it is profitable to reclaim by filling.

(*b*) Mangrove swamps adjoining the sea and separated therefrom by a small sand-bank. (See Fig. 2).

(*c*) Mouths of tidal rivers or streams flowing through flat lands, the beds being a series of pools during the dry season. Some of these rivers have low-lying swampy areas on one or both banks. (See Fig. 6.)

(*d*) Estuarine mangrove swamps, of which there is one in Trinidad. It is an extensive swamp of many square miles in area and situated at the mouth of a large tidal river with mangrove and other swamp vegetation.

5. 4 (*a*) and 4 (*d*) are not adaptable to low tide level culvert treatment, the latter having no defined sand-bank.

Description of Work on Mangrove Swamps, 4 (b).

6. The swamps were surveyed and contour levels plotted ; cross sections were taken at intervals through the sand-bank as far out to the sea as low tide level with a view of obtaining the shortest line from the deepest part of the swamp to low tide level. (See Fig. 1.)

7. In cases where the depth of the excavation through the sand-bank was not excessive it was found to be more economical to build concrete culverts with open tops and concrete sides than to adopt the enclosed box or cylinder types.

8. The open top type works satisfactorily where there is little wind or wave action. Experience taught that with the open top and a cross wind and wave of considerable strength the side wall on the windward side required raising as the culvert then acted as a groyne and eventually the sand level was raised to wall top level after which the culvert filled with sand. (See Figs. 4 and 5.)

9. With the box and cylinder types of culvert this filling in is averted, but other difficulties arise. When of small size, there is a tendency towards choking with sand and weed and other debris which cannot be readily removed by manual effort. (See Fig. 6.)

10. In order to ensure that these culverts remain clear, a sluice gate was constructed whereby with high tide and swamp empty, and low tide with swamp full, it was possible to flush either way. This worked exceedingly satis-factorily providing manipulation of the gate was carried out whenever it was necessary.

11. In cases when flushing was necessary but the flooding of the whole swamp to obtain the height of water required was not desirable, two sluice gates instead of one were constructed. One of them was placed near the sand-bank

and the other about 50 feet within the swamp. The enclosed section acted as a tank, the water being retained at high tide and released at low tide.

12. Where the location was sheltered the open-topped culverts were easy to construct. They varied in size from 2 feet to 5 feet in width with sides from 4 feet to 6 feet high. Their lengths were determined by the distance from the swamps to low tide level.
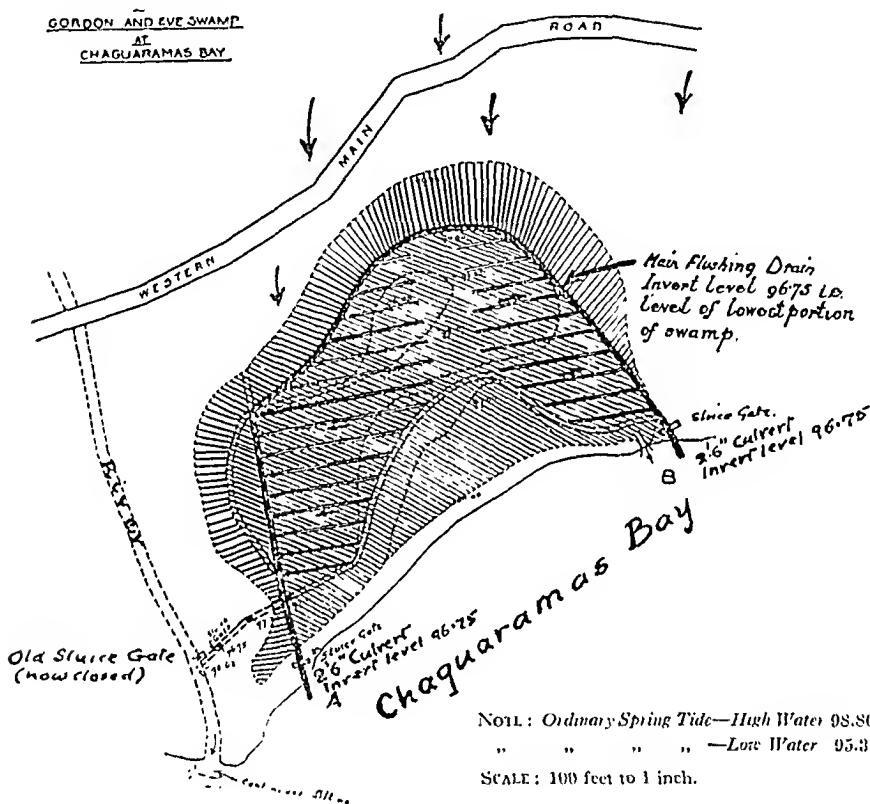
FIG. 1.—SKETCH PLAN OF SWAMP

(a) Drainage by means of main tidal flushing drain with its subsidiaries.
(b) Method of survey with contour levels.
(c) Location and construction of Low Tide Level Culverts with sluice gates.

13. The construction of box and cylinder culverts in locations where they were exposed to heavy seas was difficult. Quick-setting cement is preferable and the concrete should be heavily reinforced. On sandy foundations the culvert should be designed as a girder with piers at intervals so that when scour takes place, on an ebbing tide causing undermining, the culvert is then supported on these piers. The sea-end piers need to be of greater depth owing to the

FIG. 2.—TYPICAL MANGROVE SWAMP WITH
INTENSIVE *A. tarsimaculatus* BREEDING.

The mangroves have been recently cut
down preliminary to draining and installation
of Low Tide Level Culverts as shown on
sketch plan above.

The relation of the swamp to the sea, sand-
bank and surrounding hills is clearly seen.

FIG. 3.—HAND-OPERATED SLUICE GAT
LOW TIDE LEVEL CULVERT as seen fr
swamp at low tide.

The difference of level—*i.e.*, about 3½
between the invert of the culvert and t
of the sandbank is to be noted. The a
tidal range is 3½ feet.

FIG. 4.—OPEN TYPE OF LOW TIDE LEVEL
CULVERT with hand-operated sluice gate
viewed from the sea at low tide.

Note the banking-up of the sand against
the right wall caused by wind and wave action.
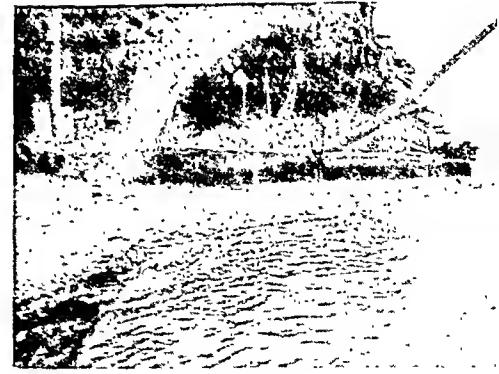
FIG. 5.—SIDE VIEW OF THE SAME CULVER
(4) at low tide.

The wall is about 4 feet high at the ga
and about 1 foot at the sea end.

FIG. 6.—RIVER MOUTH showing inlet opening of covered Low Tide Level Culvert of the box type on right bank of river.

Owing to the formation of a sandbank across the mouth of the river between periods of flood, stagnation of brackish water occurs in the river, for some distance from the sea. This permits of intensive *A. tarsimaculatus* breeding in the river during these periods.

Such stagnation and breeding is now eliminated by means of the Low Tide Level Culvert.

FIG. 7.—TYPICAL MANGROVE SWAMP, as seen from the hilly ground around the swamp after constructing two Low Tide Level Culverts—one at either end of the swamp.

The mangroves have been cut (except along the sea-shore) and the main flushing drain and the subsidiary drains have been dug as a preliminary to the planting of coconuts.

This swamp was a large permanent reservoir of *A. tarsimaculatus* which made the neighbouring villages and marine residential resorts intensely malarious. The spleen rate of a small village nearby was 82 per cent. in 1931. All anopheles breeding has now been permanently eliminated.
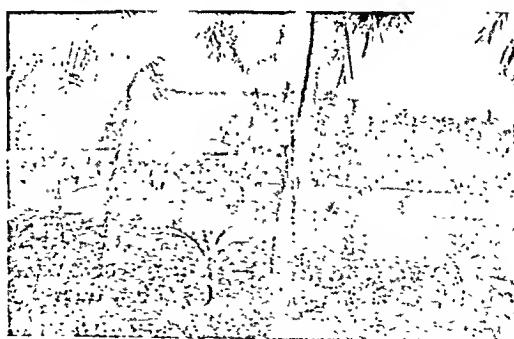
FIG. 8.—Showing agricultural and economic value of the Low Tide Level Culvert System of drainage of mangrove swamps.

The young coconut trees can be seen growing in what was once a highly malarious swamp. All anopheles breeding has been eliminated. The main flushing drain around the swamp can be seen in the foreground.

Dry coconut husks are being utilized from the neighbouring cultivation for raising the level of the swamp.

formation of deep scour holes when flood waters are being released through the culvert. Concrete cylinders in pairs sunk in well-fashion, strongly reinforced and tied, were found to be the most economical to construct. The minimum inside height of the culvert should be 3 feet 6 inches.

14. Culverts have been constructed also of precast concrete cylinders laid on rails embedded in concrete and supported at intervals on piers. Top rails were used to tie down the cylinders.

Description of Work in Connection with Mouths of Tidal Rivers or Streams, 4 (c).

15. Many rivers and streams abound which during both the wet and dry seasons have their mouths choked by sand-banks, but in the wet season these sand-banks are periodically swept away by floods. Anopheles breeding takes place on a large scale in these stagnant river mouths. It was obviously unwise to construct a low tide level culvert in the centre of the bed of the river or stream as floods would cause damage, and the upstream end of the culvert would be subject to choking. The solution was found to be in placing the upstream end of the culvert at right angles to the bed of the river and then running it in this direction to a safe distance before changing direction to the sea. (See Fig. 6.)

- Manholes were installed at convenient intervals.

Sluice Gates.

16. After experiment, the best and cheapest was found to be of the vertical slide type (penstock) operated either by an elevated roller and chain, or by wheel and worm. The handle is padlocked to prevent interference by unauthorized persons. (See Fig. 3.)

17. The size and number of sluice gates depended on the area of the swamp or the volume of water lying in the bed of the river. In the case of large areas two sluices are preferable, one at each end of the sand-bank connected by a main collecting drain carried along the inland edge of the swamp (see Figs. 1 and 7). Subsidiary drains lead into this main drain.

18. The main advantage of the low tide level culvert system lies in the fact that it is possible to drain off the whole of the swamp to low tide level as the invert of all drains can be carried through at that level. In addition, more earth (or spoil) becomes available from these drains for raising the general level of the surrounding lands for agricultural purposes. By increasing the width even additional height can be obtained. (See Figs. 7 and 8.)

19. One particular large swamp existed where the owner of the estate had reclaimed the land by deep drainage channels the earth from which he had piled up and planted coconuts thereon. This left the bottom of the drains in some cases several feet below low tide level. When the low tide level culverts were subsequently installed, they acted on the windward side as groynes collecting seaweed and sand (see Fig. 4). At high tide the sluices were opened

and the rush of sea water was utilized to carry in the seaweed and sand thrown into the culverts. By this means, the bottoms of the drains have been raised and are now firm sand.

Malarial and Agricultural Interests.

20. Mention should be made that before work is undertaken a careful study is required to find out if there are any clashing interests. The following is a concrete example :—

" A," the Medical Authority, is anxious to drain a swamp to eliminate breeding of anopheles. " B," who owns the swamp in which nothing but mangroves can grow, is desirous of re-claiming, which will result in the water level being, say, 3 feet lower than at present. " C " is the adjoining owner whose lands are, say, 3 or 4 feet above natural swamp water level and where coconuts are growing well. " C " objects to the reclamation of " B's " lands, on the ground that the lowering of the water level by this 3 feet would affect the roots of his trees, in spite of the fact that it has been suggested to him that the raising or lowering of the level of the water can be obtained, whenever desired, by the use of the sluice gates ; and, in fact, that this procedure might have a beneficial effect rather than the reverse, *i.e.*, by increased aeration of the soil and deeper root formation.

Control.

21. Definite arrangements are necessary regarding the control of the sluice gates upon completion of the work. It is essential that authority be given to some person or department to inspect frequently, and operate, the sluice gates, etc., as often as is necessary to prevent the collection of stagnant water and the breeding of anopheles. In the case of privately owned lands, close co-operation of the owners is essential.

22. It should be realised that this low tide level system was introduced primarily for anti-malarial purposes, but it has been proved that in addition to health benefits, the reclaimed lands are of definite agricultural value. Fig. 8 shows one such swamp now planted with coconuts.

23. As stated at the beginning of this appendix, conditions vary so greatly that it is not possible to give more than a very general outline of the most suitable methods of treatment. In many cases, at a small outlay, quick and permanent elimination of anopheles breeding was accomplished. In other swamps where the culverts were carried through mud of considerable depth and through mangrove roots, the work was arduous and more expensive. Heavy seas were frequently found to be troublesome.

24. The purport, therefore, of these observations is suggestive. It would be advisable first to gain experience on small and less difficult projects after which the major problems could be tackled.

RECENT EXPERIENCES OF MILD OR SYMPTOMLESS INFECTIONS WITH *TRYPANOSOMA GAMBIENSE* FROM THE GOLD COAST AND NIGERIA.

BY

W. E. COOKE,

A. L. GREGG,

AND

P. H. MANSON-BAHR,

From the Hospital for Tropical Diseases, London.

It has been recognized for some time past that a particularly avirulent strain of *Trypanosoma gambiense* is to be found in the northern parts of West Africa. Indeed, the South Nigerian trypanosome, on account of its low virulence for human beings, especially for children, led MACFIE (1913) to suggest that it should be separated as a distinct species under the name of *T. nigeriense*. He noted at that time that inoculation of this trypanosome into guineapigs resulted in the production of short, stumpy forms. WENYON (1926) remarks: As these same short forms occur in rats inoculated from human beings with undoubted *T. gambiense*, and, as the virulence of this trypanosome for man varies considerably in other parts of Africa, it is highly probable that *T. nigeriense* is merely a strain of *T. gambiense* of particularly low virulence.

One of us (P.II.M.-B.) stated, over 5 years ago, that the virulence for Europeans of strains of *T. gambiense* in certain restricted areas of the Gold Coast was becoming attenuated. (MANSON-BAHR, 1931.)

Recently LAMBORN and HOWAT (1936) have drawn attention to the fact that practically symptomless cases of human infection with what was formerly considered the particularly virulent trypanosome, *T. rhodesiense*, occur in Nyasaland natives ; and that naturally these unrecognized cases are a danger to the community as reservoirs of infection. One particular patient was discovered purely by accident and although the blood contained (for man) a massive infection, his general health, apart from co-existing bilharziasis and ankylostomiasis, remained good. This trypanosome (*T. rhodesiense*), when transmitted to dogs and rats by the syringe and through the intermediary *Glossina morsitans*, induced an acute disease in the animals. It now appears that J. B. DAVEY (1908) diagnosed the first case of sleeping sickness in Nyasaland in a native, who was in good health, without enlarged glands ; and who had been living for over a year in a fly-free area maintaining good physique and appearance. It was formerly considered that Europeans were specially susceptible to trypanosome infection and, when naturally infected, invariably developed fever, enlarged glands, a rash and other objective symptoms of the disease. Certainly this has been our experience of a series of fifty-nine European patients seen at the Hospital for Tropical Diseases during the last 16 years.

Now SICÉ, ROBIN and MERCIER (1936) have recorded the case of a European who left the Ivory Coast for France and remained in good health for nearly a year before he complained of ill-health (see Case 4). MOUSTARDIER (1936) has recorded that a native of the Ivory Coast developed signs of trypanosomiasis 2 years after going to reside in Madagascar, and eventually died of trypanosomal meningo-encephalitis. DUREN and VAN DEN BRANDEN (1934) have also remarked upon European cases from the Belgian Congo of singularly slow evolution. In the case of one patient, it was 25 months before the diagnosis became clear in Belgium. RIOU and MOYNE (1933) found a case of a young native girl in Dakar town who had taken 8 years to develop clinical signs of the disease.

We are now in a position to record three consecutive cases which have been diagnosed in the ordinary routine of practice during the last year, and one earlier case.

It is admitted that the detection of these cases was rendered possible only by our habit of making routine blood examinations of everyone who returns from the West Coast of Africa for possible blood infections of all kinds. One case showed no apparent evidence of disease and stated he felt quite well ; another was discovered to have on his shoulder the characteristic circinate rash (see Figure), whilst the other two gave histories of otherwise unexplained fever while on the Coast. It is to be noted also that in two cases the local reaction caused by the infected tsetse bite, the so-called trypanosome " chancre " (GRAF, 1929), was still visible on careful examination, and that the blood infection

was a particularly heavy one and, therefore, easily diagnosable under the microscope.

The following is the epitome of the cases with the results of blood inoculations into animals. Special note should be made of the vicarious appearance of the trypanosomes in the blood stream of man and the lack of virulence of these organisms for laboratory animals.

CASES.

Case 1.

F.J.R., mining engineer, aged 36, was examined by A.L.G., on behalf of P.M.-B., on 14th August, 1936, with reference to his fitness for service as an inspector of mines, Gold Coast. At the time of the examination he appeared to be in excellent health, but a typical semicircular trypanosome rash, 2 inches in diameter and ½ inch wide, was noticed over the left scapula (see Figure). He gave a history of having served on the Gold Coast from December, 1924, to February, 1926, and again from February, 1935, to June, 1936. Except for several attacks of malaria, he had kept well till January, 1936. On 13th January, at O'Buom, near Bukwai, 100 miles from Accra, he was bitten on the right calf by a tsetse fly and that same evening his temperature rose to 104° F. He felt ill for 1 month afterwards with generalized backache, pain in the knees and fever, thought to be malaria. He was treated by quinine injections and had no further fever after a month's treatment.

CASE 1.—TRYPANOSOMIASIS RASH.

On admission to the Hospital for Tropical Diseases on 14th August, 1936, the spleen was just palpable, a large " bruise " was noticed on the dorsum of the right calf at the site of the tsetse bite and a few shotty glands were present at the back of the neck. A palpable gland was present in the right axilla and, in addition to the rash on the left scapula, a circular faded rash was perceptible on the back and chest ; the temperature was normal ; bloodfilms showed trypanosomes in fairly large numbers (two trypanosomes to one hundred leucocytes).

The patient was treated with Bayer 205 in 1 gramme doses at 5-day intervals intravenously (10 grammes in all). He was also found to be infected

with *Entamoeba histolytica* and treatment with emetine bismuth iodide and yatren was carried out concurrently with the Bayer treatment. A faint cloud of albumin appeared in the urine on 28th August, and granular and hyaline casts were seen for the first time on the 31st. The fourth injection of Bayer 205 was followed by a reaction, temperature 101° F. On re-examination on 26th October, casts were still present, otherwise the patient was perfectly fit. Rats inoculated with blood on 14th August (Col. F. P. MACKIE) have remained uninfected.

Case 2.

R.J.P.H., mining engineer, aged 46, was sent to W.E.C. on 15th September, 1936, to be passed fit for service in British Guiana. He had served in Nigeria from 1912 to 1929, and on the Gold Coast from 1929 to 1936. Most of his service was spent near Dunkwa at the junction of the Offin and Pra Rivers, Gold Coast. Except for occasional attacks of " fever " he kept well and returned to England in March, 1936. With the exception of one attack of " malaria " which he thought he had on arrival, he had kept in good health since he had been in England. Examination revealed nothing abnormal save a slight enlargement of the spleen and he was passed as fit. A bloodfilm taken as a routine procedure revealed a fairly heavy trypanosome infection (one trypanosome to about every twelve fields of the 1/12th). The patient, who had gone to Cornwall, was recalled and was seen again on 18th September, when wet and stained bloodfilms examined for 1½ hours were negative for trypansomes which have not again appeared.

On admission to the Hospital for Tropical Diseases on 18th September, 1936, his temperature was normal and the spleen was just palpable, the central nervous system was normal and no enlarged glands were found. A pigmented area 1½ inches in diameter on the left ankle was considered by the patient to be the site of a tsetse-fly bite sustained early this year. He volunteered the information that he had had a " rash " in February which disappeared. Inoculations of blood (Wellcome Bureau of Scientific Research) into rats, mice and monkey proved negative.

Treatment consisted of 1 gramme doses of Bayer 205 given intraveneously (10 grammes in all) ; the first three doses were at 5-day intervals and the remainder every 7 days. Albumin and casts were present in the urine after the second injection and some conjunctival hyperaemia appeared after the sixth, but this was attributed to excessive reading whilst in Hospital. Otherwise the patient kept quite well throughout treatment.

Case 3.

Miss E.G., a missionary, aged 52, had lived in Nigeria on and off since 1921. Except for appendicectomy in 1927 and slight attacks of malaria, she had kept well. In September, 1935, she returned to Nigeria from leave and had

three or four attacks of " fever." On June 8th, 1936, she went up-country to Igumale, 200 miles north of Port Harcourt, and was bitten by a tsetse fly " behind the right knee." A week afterwards she noticed an area of hyperaemia at the site of the bite which gradually spread till it became the size of the palm of the hand and was of a dark purple colour. About 3 weeks after the bite she noticed a rash " like measles " over her back, chest and abdomen. On 20th June, she got " fever " and on the 22nd a rigor occurred with temperature 104° F. She was given quinine and the temperature came down, but later she complained of " nerve pains over her body." A week later fever returned and she was admitted to hospital at Port Harcourt where, on account of a positive Widal, she was diagnosed and treated as a case of paratyphoid. After discharge from hospital the fever returned and she was sent back to England. On 8th, 9th and 10th October, she had further attacks of fever and a bloodslide sent to the Hospital for Tropical Diseases showed trypanosomes in fair numbers. She was admitted to the hospital under the care of W.E.C. on 16th October, 1936.

On admission her temperature was normal and her condition fair. The spleen was just palpable, and there was a definite hyperaemic area over the posterior aspect of the right knee. The glands in the left cervical area were palpable and a typical circinate rash was present over the back, chest and abdomen. Treatment with Bayer 205, in 1 gramme doses intravenously was instituted. A faint cloud of albumin and a few granular casts were present in the urine on 28th October, 8 days after commencement of treatment. The serum agglutinated typhoid to 1 : 250, and paratyphoid-A. 1 : 25. Rats inoculated with the patient's blood at the time of admission to hospital (Wellcome Bureau of Scientific Research) acquired a trypanosome infection. A full course of 10 grammes of Bayer 205 has been given.

Case 4.

J.C., aged 40, had spent 18 years on the Gold Coast (Accra). He returned to England on retirement in June, 1929.

For 1¼ years he had noticed a loss of energy ; then in March, 1929, irregular fever commenced which came on 21 days after a definite tsetse bite on the left ankle. Following the bite, local swelling occurred at the site ½ inch in diameter, and later on an erythematous patch with a bluish centre appeared ; the groin glands also became enlarged. Quinine had no effect on the pyrexial attacks. In Africa, the blood had been tested and he was told he had an enteric infection (March, 1929).

On arrival in England, his health gradually deteriorated. He was rarely 10 days without fever, the neck glands began to swell and a circinate erythematous rash occasionally appeared on the abdomen, especially after a warm bath.

He was seen in the out-patient department of the Hospital for Tropical Diseases on the 3rd of July, 1930, *over a year after his arrival in England.* He then had enlarged glands in neck, axillae and groins, definitely enlarged spleen,

a typical erythematous half-circle rash on left side of abdomen, pyrexia and light secondary anaemia. No symptoms of central nervous system involvement were present. He was at once admitted to hospital. *Trypanosoma gambiense* were present in blood and in gland puncture fluid marked agglutination of red blood cells was noted. The cerebrospinal fluid gave a pressure of 200 mm. and contained 35 cells per c.mm; there was globulin in excess and scanty trypanosomes were present.

Blood and cerebrospinal fluid inoculated into rats (Wellcome Bureau of Scientific Research) failed to produce any infection.

Treatment was commenced with Bayer 205 (total 2 grammes) followed by tryparsamide (total 23 grammes). Lumbar puncture on 14th August, 1930 showed fluid and cell count normal. This patient reported again in January, 1932. Blood and cerebrospinal fluid were examined. No evidence of trypanosomiasis was found.

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THE DEVELOPMENT OF MOSQUITOES IN COMPLETE DARKNESS.

BY

B JOBLING,

Wellcome Entomological Field Laboratories, Wellcome Bureau of Scientific Research, London.

INTRODUCTION.

It has already been mentioned in my previous paper[*] that the autogenous *Culex pipiens* can develop perfectly, mate and lay fertile eggs in complete darkness. Since the publication of that paper I have made the same experiments with the common, nonautogenous *Culex pipiens, Culex fatigans* and the yellow fever mosquito *Aëdes argenteus.*

It is the purpose of this paper to give the description of the technique which has been used and the results of these experiments.

EXPERIMENTS.

The experiments with the above-mentioned mosquitoes were carried out in an apparatus which was made of thick cardboard (Fig. 1). The walls of this

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[*]Jobling, B. (1935). The effect of light and darkness on oviposition in mosquitoes. *Trans. R. Soc. trop. Med. & Hyg.*, xxix, 157.

apparatus were 12 inches high and 7 inches wide. The inside dimensions of it were adapted for the accommodation of an enamelled dish, 6 inches in diameter, which was used for the breeding of the mosquitoes. The three walls of this apparatus extended about 2 inches below the bottom, which was provided with a ventilating hole (*o.*) in each corner. Each ventilating hole was screened below the bottom by a U-shaped piece of cardboard (*u.p.*) attached to the bottom and to the wall. Inside the apparatus each of these holes was screened by an obliquely fixed, oblong plate (*p.*). In the middle of the top of the apparatus there was a round ventilating hole, 2 inches in diameter. Inside the apparatus this hole was screened by a disc (*d.*) which was attached under it to the top by two wooden blocks, $\frac{2}{5}$ inch long. On top it was surrounded by an inner cylinder (*i.c.*) of slightly bigger diameter. This had two semicircular holes (*o.*) in the upper part and to its rim was attached a disc (*d'.*) which screened the interior from above. The inner cylinder was surrounded by an outer cylinder (*o.c.*). This was about 2 inches longer than the inner one and of such a diameter that its wall was separated by $\frac{3}{10}$ inch from the edge of the upper disc. To prevent the escape of the mosquitoes through the ventilators, they were covered inside the apparatus with mosquito netting (*m.n.*).

The door (*do.*) was made of a wooden board to which was fixed a wooden frame (*w.f.*). Between this frame and the edge of the door the board was lined with a thick woollen material. When the apparatus was closed, the frame of the door rested in the wooden frame of the apparatus and was in contact with a narrow screen (*s.*).

For aerating the infusion in the dish, the apparatus was provided with an aerating pipe (*a.p.*). This pipe was inserted through a cork which was fixed in the wall and was connected by rubber tubing with an air pump. In order to exclude completely the passage of light into the pipe, between the end of the rubber tubing and the cork, the latter had an excavation round the pipe to receive the end of the rubber tubing. The temperature of the air and water was measured by two thermometers. The projecting part of the air thermometer was screened by a light-proof case inside the apparatus, whereas the water thermometer was screened from outside. All the internal parts of the apparatus were painted black, while externally it was given two coats of white enamel.

The light-proof construction of the apparatus was tested by the following method :—In a dark room a photographic plate was cut in two halves. One half was immediately developed and fixed, whereas the other half was put inside the apparatus, which was closed and placed in front of a window. After a week the apparatus was brought into the dark room, the plate was taken out, developed and fixed and then compared with the other half. On comparison of these two halves it was found that there was no difference in their colouration, both being quite clear. This indicated the absence of light in the apparatus when exposed to daylight.

The liquid for the breeding of the mosquitoes was dog biscuit infusion in all the experiments, because it has been found that the conditions in this remain constant for a much longer period of time than in hay infusion.

The infusion was prepared a day before and then strained into two dishes of the same size. Into each dish was introduced a raft of eggs. These were always of the same age and contained the same number of eggs. In the experiments with the nonautogenous *Culex pipiens* and *C. fatigans* one big raft was divided into two equal parts. After the introduction of the eggs, one dish was placed in the apparatus, while the other was left exposed to light.
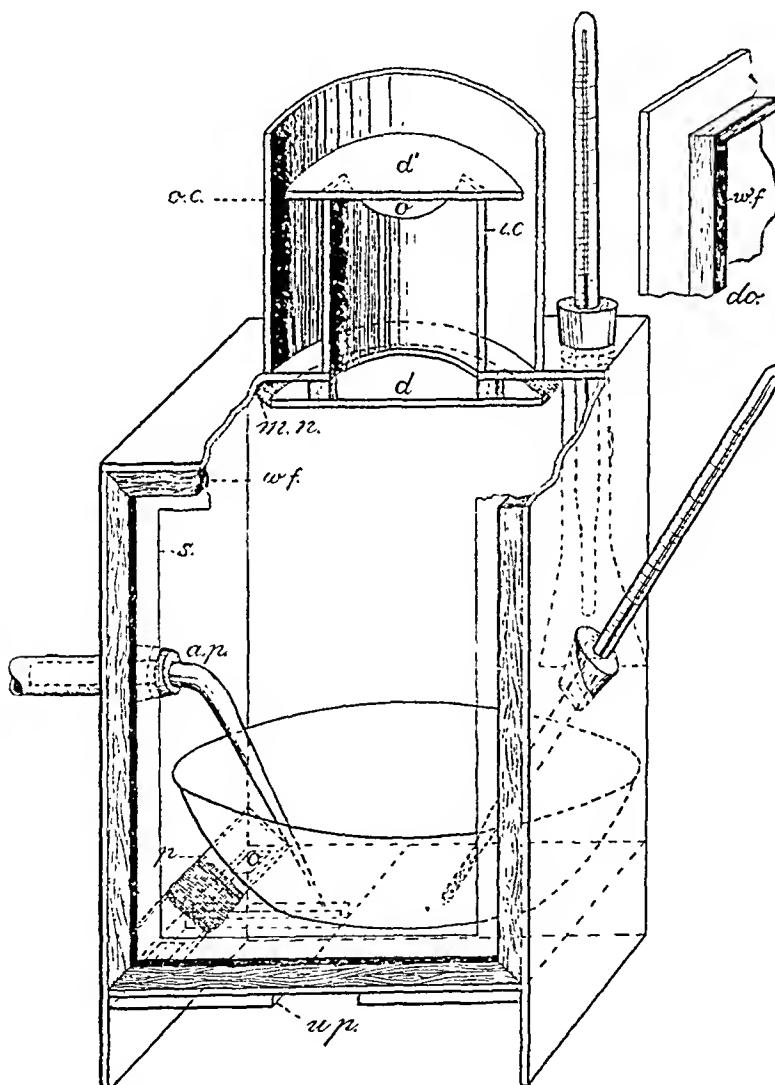
FIG. 1.

APPARATUS FOR BREEDING MOSQUITOES IN ABSENCE OF LIGHT.

The breeding in the exposed dish has not been considered as a control in these experiments, because it is impossible to maintain exactly the same conditions in the two separate dishes with the same infusion. It has often happened during the breeding of the mosquitoes that in one dish all the eggs develop to the adult stage, whereas in the other dish, with the same infusion, the eggs from the same raft develop normally only to the first or the second stage larvae, and then they begin to die. In this case the mortality greatly increases when the remaining larvae reach the fourth stage, so that none of them develop to adults. This mortality of the larvae is produced by the action of the bacteria in the infusion. Sometimes the sudden dying of the larvae begins together with a change of colour of the infusion and a slight change of the pH. The dog biscuit and the bread infusions change from milky to brownish colour and become much more transparent. But sometimes there are no obvious changes in the infusion when the larvae begin to die, and the pH remains the same from the moment of the introduction of the eggs up to the appearance of the fourth stage larvae.

The breeding in the open dish was used as an indicator of the end of the experiment, and the apparatus was always opened and examined 1 or 2 days after the hatching of the last mosquito from the open dish. With each species of mosquito the experiment was repeated. As it was impossible to avoid the introduction of diffused light into the apparatus when the pH and the temperature of the infusion were taken, the second experiment was performed without the reading of these factors. In all the experiments the pH of the infusion in both dishes was always the same, and its changes occurred simultaneously in both dishes, with the exception of one experiment, where for 2 days the difference was 0·5. The pH of two of the experiments is shown in the chart together with the temperature of the infusions and the air temperature (Fig. 2).

As regards the development of the mosquitoes in the two dishes, the temperature of the infusion was the most important factor. Since in all the experiments the temperature of one dish never varied more than 1° C. from that of the other, and the mean temperature was practically the same in both, this factor will not be given for each experiment separately.

THE DEVELOPMENT OF THE MOSQUITOES.

The Common Nonautogenous *Culex pipiens*.

In the experiments with this mosquito the infusion in both dishes was equally aerated and an equal number of eggs from the same raft was introduced into each dish. The first experiment, when the temperature and the pH were taken, was not successful. Out of 80 eggs only 11 developed to the adult stage in the dish exposed to light. The greatest mortality in this dish occurred amongst the fourth stage larvae and the pupae. In the dish in the apparatus all the larvae died in the third or the fourth stage.

The experiment was repeated without taking the temperature and the samples of infusion for the pH. At the end of the experiment 36 adults emerged from the dish ·exposed to light and 23 dead pupae and 14 dead fourth stage larvae were found in the infusion. In the apparatus 24 adults, 6 dead pupae and 14 dead fourth stage larvae were found. The result of the last experiment indicates that the nonautogenous *C. pipiens* can develop in complete darkness. The small percentage of development (30 per cent.) was not due to the absence of light, but to the unfavourable conditions in the infusion. The larvae of this mosquito are more sensitive to changes in their environment than are those of the other mosquitoes used in these experiments.
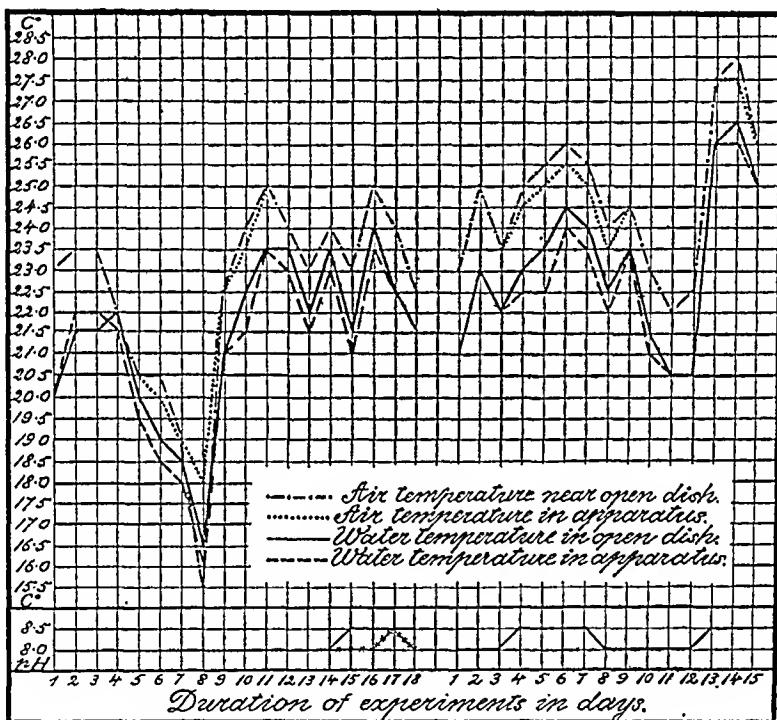
FIG. 2.

GRAPHS SHOWING VARIATION OF pH, TEMPERATURE OF INFUSION AND AIR TEMPERATURE IN TWO EXPERIMENTS.

The Autogenous *Culex pipiens*.

In the experiment with this mosquito the infusion was equally aerated in both dishes, and a separate raft of eggs was introduced into each. These rafts had the same number of eggs and were laid at the same time. During the first two experiments the pH, the temperature of the infusion and the air temperature were taken every day. In one of these experiments 51 eggs produced 46 adults in the dish exposed to light, while from the same number of eggs there were

found 47 adults and 2 live pupae in the apparatus. In the other experiment out of 53 eggs in the open dish, 45 developed to the adult, whereas in the apparatus the same number of eggs produced 47 adults, and three new rafts of eggs were found from which numerous first stage larvae had already hatched.

In order to exclude the light completely the experiment was repeated without taking the temperature and the pH. In this experiment 36 eggs were introduced into each dish. Twenty-four days after the introduction of the rafts, the breeding in the open dish was finished, and out of 36 eggs, 29 developed to the adult. The apparatus was opened 16 days after the emergence of the last mosquito from the open dish, and in it were found 15 dead adults, 10 live and 9 dead fourth stage larvae and 22 pupa cases. As regards the adults and the pupa cases, they were undoubtedly of the first generation, but it is impossible to know definitely the exact number of the former, because their dead bodies and the empty pupa cases usually disintegrate very quickly in the infusion. All the fourth stage larvae must have belonged to the second generation, because at the mean temperature 19·4° C. in the room during the experiment, each stage of the larva lasts only 3 or 4 days. A few larvae may prolong their development, but this retardation indicates their unhealthy condition. Such larvae rarely develop to the adult and generally die during pupation. The presence of only a small number of larvae of the second generation, was due to the deficiency of food and the unfavourable conditions in the infusion, which was found to be clear and brownish in colour.

The results of these experiments show quite conclusively that this mosquito can develop, mate and produce a second generation in complete darkness.

Culex fatigans.

In the experiments with this mosquito the infusion was not aerated and eggs of the same raft were used for both dishes. The first experiment, when the temperature and the pH were taken, gave the following result. Out of 70 eggs placed in the dish exposed to light, 57 produced mosquitoes, while in the apparatus the same number of eggs produced 67 mosquitoes. In the other experiment, when the pH and the temperature of the infusion were not taken, the result was practically the same. Out of 75 eggs placed in the dish exposed to light, 58 developed to the adult, while in the complete darkness of the apparatus, the same number of eggs produced 63 adults.

The following experiment was made in order to see whether this mosquito can lay eggs in complete darkness. Six females were put into the apparatus after they had been fed. Four days later the apparatus was opened and in the dish were found six rafts of eggs from which numerous first stage larvae had already hatched.

The above described experiments show quite conclusively that *Culex fatigans* can lay eggs and breed perfectly in complete darkness.

Aëdes argenteus.

In the experiments with this mosquito the infusion in the dishes was not aerated. As the females of this mosquito rarely produce a large number of eggs at one laying, in the first experiment, when the temperature of the infusion and the pH were taken, each dish received 70 eggs which were laid at the same time, but by different females. In the dish exposed to light, out of 70 eggs, 47 produced adults and there were present several unhatched eggs, 3 dead fourth stage larvae and 5 dead pupae. In the apparatus the same number of eggs produced 38 adults and 6 dead third stage larvae, 2 dead fourth stage larvae and several unhatched eggs were found in the infusion.

During the second experiment neither the temperature of the infusion nor the pH were taken. From 50 eggs laid by one female in the dish exposed to light, 45 developed to the adult, while in the apparatus, from the same number of eggs, which were laid by another female, 45 developed to the adult and in the infusion were found 3 live fourth stage larvae and 2 dead pupae.

The results of these experiments show that Aëdes argenteus can develop perfectly in complete darkness. The following experiment shows that this mosquito can also lay eggs in complete darkness. Six fed females were put into the apparatus. When 4 days later it was opened 450 eggs were found in the infusion. That all the six females took part in the laying of these eggs was shown by the examination of their abdomens.

Conclusion.

The results of the experiments show quite conclusively that the common, nonautogenous Culex pipiens, the autogenous Culex pipiens, Culex fatigans and Aëdes argenteus can develop perfectly in complete absence of light. The ability of the larvae of these mosquitoes to exist under this condition is probably due to their feeding habit. They are omnivorous and are not affected by the absence of algae, such as Cyanophyceae and Chlorophyceae and the high aquatic plants, which cannot exist without light. In complete darkness the food of these larvae consists of small particles of organic matter, and of such organisms as bacteria, some Mastigophora and Ciliata. The existence of these organisms depends also upon the presence of organic matter in the water and they also are not affected by the absence of light.

The experiments also show that Culex fatigans, Aëdes argenteus and the autogenous Culex pipiens can lay eggs in complete darkness and that the last named mosquito can produce a second generation. As regards the oviposition, it has already been pointed out in my previous paper (JOBLING, 1935) that in complete darkness it is governed either by the chemical condition of the water, or by the water vapour, or by the combined action of these two factors, because in complete darkness there is no stimulus to develop the phototactic reaction in the mosquito. The same can be said about the mating. The male cannot

see the female in complete darkness; therefore, it is attracted to it either by the scent, or the sound which the female may produce, or by both of these factors.

The results of these experiments may have some practical interest when we remember that suitable breeding places may be present in completely dark situations. The larvae of some of these mosquitoes have been found in dark cellars, in dark interiors of cisterns and in tanks built inside walls. Such places represent more dangerous mosquito breeding grounds than those in the open, especially as regards the urban species, because breeding in these places is not always easily detected.

Summary.

The experiments were made with two races of *Culex pipiens*, *Culex fatigans* and *Aëdes argenteus* in a specially constructed apparatus.

The results of these experiments show quite conclusively that all these mosquitoes can develop perfectly in complete darkness.

Aëdes argenteus, *Culex fatigans* and the autogenous *Culex pipiens* can lay eggs in complete darkness and the last named mosquito can mate and produce a second generation.

The ability of the larvae of these mosquitoes to develop perfectly in absence of light is probably due to their feeding habit. They are omnivorous and can exist upon organic matter, and upon such organisms as can also live in complete absence of light if organic matter is present in the water.

RUBBER INTRODUCED AS LATEX CAUSING PATHOLOGICAL CONDITIONS IN THE BELGIAN CONGO.

BY

CLEMENT C. CHESTERMAN, *O.B.E.*, M.D., M.R.C.P.

The continual discovery of new uses for rubber sometimes renders necessary its forcible extraction from unwilling natives, as the following two cases indicate.

Case I.

A native woman, aged 45, came to the Yakusu Hospital of the Baptist Missionary Society, Belgian Congo, complaining of dyspepsia and a " lump in the stomach." Abdominal palpation revealed a sausage-shaped epigastric swelling, smooth and regular in outline and freely movable. Adjacent to its

€. COAGULATED RUBBER CAST OF THE STOMACH—REMOVED BY GASTROTOMY.
(About half actual size.)

right end was a smaller swelling of similar shape and mobility. There was little tenderness but some wasting. A tuberculin test and an examination of the stools for ova of schistosomes proved negative and anthelmintic treatment failed to produce a ball of ascarids, which it was thought might be present. Had she been a neurotic young girl the possibility of a hair ball might have been entertained although it is to be doubted whether the short frizzy hair of the negro would

lend itself to this formation. Laparotomy was performed under spinal anaesthesia, and gastrotomy revealed the two lumps, which were strung together like sausages, as being composed of a homogenous elastic yellow material, actually, crepe rubber. The larger, 11 cm. × 4 cm., and the smaller, 7 cm. × 2½ cm., weighed together 140 grammes.

The husband explained that his wife had been troubled with worms and on his advice had three times drunk about ½ pint of latex, the milky sap of the wild rubber vine. The first two resultant cords had been passed in due course but he was disappointed in not finding entangled in their substance the offending worms. The third time the free acid of the stomach must have coagulated the latex which formed itself into an exact case of that organ showing perfectly the gastric and pyloric segments. The patient made an uninterrupted recovery, and signed the pledge to drink no more rubber.

Case 2.

An old woman was brought into hospital with a history of chronic dysentery. Faecal examination revealed the presence of *E. dysenteriae* and ova of our local variety of intestinal schistosome, *S. intercalatum* (FISHER, 1934). The symptoms of peritonitis were present and necropsy next day revealed a green and gangrenous sigmoid colon. On opening this organ an irregular cast of its lumen was found in coagulated rubber. The patient's friends admitted the administration a week previously of an enema of latex.

There was at one time considerable talk of Congo rubber atrocities but it would appear that it is now one's duty to protect the natives from their own passion for rubber. I have extensive evidence that this rather elastic habit of oral, rectal and vaginal (the last as an abortifacient) administration of latex is becoming widespread among Congo natives, frequently with fatal results in children.

NOTES ON FILARIASIS AND ITS TRANSMISSION BY MAURITIAN ANOPHELINES.

BY

S. GEBERT,

Entomologist, Medical and Health Department, Mauritius.

The establishment of an Entomological Research Laboratory in Port Louis, in the yard of the Civil Hospital, which is due to the enterprise of the Medical Director, and the proximity of anopheline breeding grounds has enabled investigations in the incidence of filariasis and its transmission by mosquitoes to be carried out under favourable conditions.

In 1928 KIRK described the results of a filarial survey carried out by him in Port Louis. He visited houses between 8.30 p.m. and midnight and took

blood specimens from 658 persons who gave a filarial infection rate of 11·2 per
cent.   He noted also that the infection rate was lower amongst females than
amongst males.   Commenting on the work of D'EMMEREZ, DE CHARMOY and
the late DARUTY DE GRANPRÉ (1900), who had proved that *Culex fatigans* was a
vector, KIRK observes that it still remains to be discovered whether that mosquito
is the sole vector, or whether other local species also play their part.   The
results recorded in this paper offer a partial answer to this remark.

INCIDENCE.

Blood films from 1,683 persons admitted to the Civil Hospital have been
examined with the following results :—

	Males.		Females.	
	Examined.	Percentage Infected.	Examined.	Percentage Infected.
Port Louis	801	9·1	400	3·0
Other districts	386	11·6	96	3·1

It will be seen that while Port Louis gives a percentage of 9·1, a higher
figure is obtainable for other districts of which certain localities seem specially
infected.   Out of sixty-three persons from Pamplemousses, ten were infected
(16·8 per cent.).   In the Black River district, Les Bambous gave an infection
rate of 28 per cent. and Petite-Rivière one of 24 per cent.   The Flacq district
gave a rate of 26 per cent., while that of Rivière-du-Rempart showed three
positive cases out of thirty-one persons examined (9·7 per cent.).

In the district of Port Louis itself the regions which produced the greatest
number of cases were :—

Grand-River-North-West (28 per cent.);   Les Cassis (28 per cent.);
Roche-Bois (22 per cent.);   Belle-Village (21 per cent.).

In marking the cases of infection on a plan of the town it soon became evident
that these were invariably in the vicinity of streams or open drains.   It was
clear that in Port Louis filariasis follows the water courses.

The figures so far obtained indicate that filariasis is well established all
over the island, and there would appear to be no reason why the cases should
not increase in number from year to year.

The figures obtained do not show the actual infection rate for there is a
definite periodicity, filarial embryos disappearing from the circulation from time
to time for periods of several days' duration.   Furthermore, slight infections are
easily overlooked.   The season of the year also appears to influence the occurrence

of embryos in the peripheral blood. These are generally more numerous in the cool season, with the result that a higher percentage of cases is detected at this time of the year. Another effect of this variation is the greater transmission in winter in the coastal belt where the temperature does not fall low enough to produce a decrease in the number of anophelines.

A puzzling fact, one for which no satisfactory explanation can be offered, is the lower infection rate amongst the female population.

Mosquito Experiments.

Experiments were carried out with anopheline mosquitoes with a view to determining the possibility of their being vectors. For that purpose four species were employed, all of which were hatched from larvae in the laboratory. The mosquitoes were enclosed singly or to the number of two or three in flat-bottomed specimen tubes covered with mosquito netting, through which they fed when the tube was applied to the patient's skin. As far as possible feeding was carried out on patients showing about 150 embryos in a thick film (about 0·1 c.c. of blood). After feeding, the mosquitoes remained in the tubes, to the mouth of which a wet plug of cotton wool, smeared with honey, was applied daily. The dissections were made under a low power binocular microscope. This enabled the embryos separated from the tissues to be studied and counted. Permanent preparations of the developing embryos were made by removing most of the liquid from the slide, allowing it to become nearly dry and then fixing the specimen in Schaudinn's fluid. The film was then washed, stained with Delafield's haematoxylin and finally mounted in Canada balsam according to the usual technique for sections.

The experiments have shown that *Anopheles costalis*, *A. funestus* and *A. maculipalpis* can all be experimentally infected and that in them the embryos complete their development. Complete development was obtained in the first two of these in 16 days. The cycle in *A. maculipalpis* probably requires a longer period as filariae were found in the proboscis only after 19 days. In one mosquito, dissected on the 16th day of infection, the filariae were still in the thorax. With the other two species variations from the 16-day period occur. In all, seventeen *A. costalis*, four *A. funestus* and four *A. maculipalpis* were fed on patients ; and, in all of them, one or more developmental forms were found, the actual stage depending on the time of dissection after feeding. As regards *A. mauritianus* this would not appear to be a vector, since of thirteen specimens fed on cases (some of which at least led to infection in the other mosquitoes), only two showed embryos which had reached the thick stage. In one of these, the thick embryos had evidently died and were degenerating when the dissection was made. During the experiments the mosquitoes were kept at temperatures varying from 17·7° C. to 32·7° C. with an average of about 20° C.

The process of development in the mosquito calls for no special remarks as

it follows the well-known development which has been described as occurring in other mosquitoes.

CONCLUSION.

1. In Mauritius, filariasis due to *Wuchereria bancrofti* occurs throughout the island.

2. In addition to *Culex fatigans* three species of anopheles (*A. costalis*, *A. funestus, A. maculipalpis*) are all capable of acting as vectors.

3. *A. mauritianus* is unable to do so.

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CORRESPONDENCE.

BERIBERI IN EGYPT.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

In his recent paper on beriberi in Egypt, Dr. DAY* clearly describes cases of general oedema not associated with cardiac or renal disease, but with certain endemic affections, especially cases resembling beriberi of the oedematous form; in each instance the signs of pellagra were present and had preceded the oedema. It is interesting to note, after this interval of time, that medical officers who were in charge of the Turkish prisoners of war in Egypt, especially at the hospital in Heliopolis in 1916, were puzzled with the same chain of events. These cases were studied by A. D. BIGLAND, J. I. ENRIGHT and myself, and we were much struck by what one may call a " mixed syndrome." Having seen a good deal of oedematous beriberi prior to the war, I was at first inclined to believe that we were dealing with an extensive outbreak of beriberi caused by diet deficiency. There was general anasarca, hyperaesthesia of the muscles and loss of deep reflexes in some; and it was not till the oedema had cleared away and the rash had appeared, and a considerable number of autopsies had been performed, that we began to recognize that this was but a transient phase in the somewhat varied clinical picture of pellagra. Indeed, I still believe that we were observing on a large scale a combined clinical picture of both these deficiency diseases. From the point of view of the pathologist it was especially interesting to note at autopsy that in nearly all these cases abundant evidence of chronic bacillary dysentery was found in the large intestines, some had pulmonary tuberculosis, as well as chronic malaria and ankylostomiasis. In some, too, haemorrhages into the suprarenal glands could be demonstrated together with brown atrophy of the heart. There is nothing I regret so much as that, owing to the exigencies of service, all the pathological specimens and most of the notes of my investigations were destroyed, whilst I was in Palestine. A very good description of the pellagra outbreak in Egypt amongst the Ottoman prisoners of war is given by A. D. BIGLAND in the *Lancet*, 1920, i, pp. 946-953. Oedema was noted in 81 out of 232 cases. This account, together with the laboratory investigations may, I think, be considered one of the most thorough enquiries into this disease made at that period; and certainly the conclusions reached concerning the biological protein value of the food are closely in accord with the popular theories of the etiology of pellagra at the present day.

Together with this account of pellagra, BIGLAND's studies of oedema as a symptom of so-called food deficiency diseases (*Lancet*, 1920, i, pp. 243-247) must be considered, as also a further account of war oedema in Turkish prisoners of war by J. I. ENRIGHT (*Lancet*, 1920, i, pp. 314-316).

I am, Sir, etc.,

PHILIP MANSON-BAHR.

*DAY, H. B. (1936). *Trans. R. Soc. trop. Med. Hyg.*,, xxx (3), 345.

THE BUTTERFLY SIGN AND TONGUE PIGMENTATION.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

I was interested in McGREGOR's article on the significance of the butterfly sign and of tongue pigmentation in these TRANSACTIONS.*

I was under the impression that the butterfly sign was considered, in the old days, a sign of intestinal tuberculosis. This is the first time that I have heard of its being traced to schistosome infection (SINDERSON) and to urinary infection. Schistosome infestation is unknown in South India and yet the butterfly sign is common enough.

As for tongue pigmentation it is difficult to appreciate how the tongue and especially how the palate (for the palate is also pigmented in many of these cases) is accessible to solar radiation. We felt that the tongue pigmentation was due to the hookworm toxin, as McGREGOR did in the first instance, and also to the Leishman-Donovan body of kala-azar. McGREGOR's analysis of stools in persons with pigmented tongues seems to dispose of the hookworm theory. His analysis is, however, admittedly incomplete. One criticism of his remarks regarding urinary infection might be that it would be more appropriate to find out the percentage incidence of tongue pigmentation in known urinary infection, rather than to look for evidence of urine infection in persons with tongue pigmentation.

It strikes me that tongue pigmentation in the tropics, as elsewhere, may be due to the toxic depression of the adrenal cortex from any cause. The adrenal cortex presides over sodium and presumably over Vitamin C metabolism. I recently came across the suggestion (by McCANCE I think) that pigmentation in Addison's disease was probably due to deficiency of Vitamin C, as experimentally sodium deficiency never produced pigmentation, but did produce practically all the other symptoms of Addison's disease. Is pigmentation, then, a measure of sub-clinical deficiency of Vitamin C? It should not be difficult to check this hypothesis by the new method of finding out how much of a large test dose of the vitamin is recovered from the urine.

I remain, Sir, etc.,

Madras Medical Service,   S. K. SUNDARAM.
Government General Hospital.
Madras.

*McGREGOR, I. J. (1936). The significance of the butterfly sign and of tongue pigmentation. *Trans. R. Soc. trop. Med. & Hyg.,* xxx (2), 229.
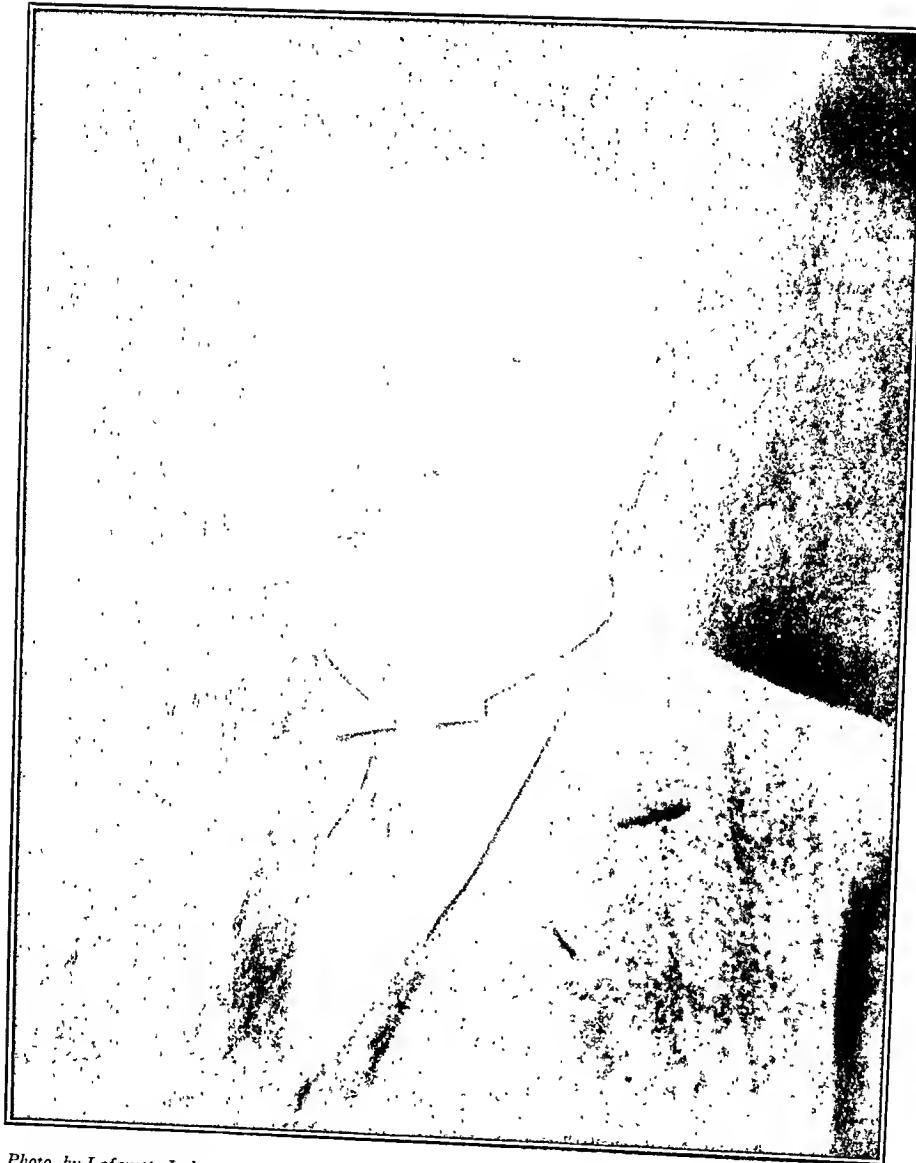
Photo. by Lafayette Ltd.

OBITUARY.

SIR ARNOLD THEILER, *K.C.M.G.,*
DR. MED. VET., D.V.SC., D.SC., PH.D.

The death in London on July 24th, 1936, at the age of 69, of Sir ARNOLD THEILER, Honorary Fellow of the Royal Society of ·Tropical Medicine and Hygiene since 1926, marked the passing of a great scientist, a world-famous veterinarian and a compelling personality.

The reputation of Sir ARNOLD was made in South Africa and from the date of his entry into the Transvaal in 1891 his life was an arduous but triumphant struggle against the many diseases which had thwarted the agricultural development of the country. As Sir JOHN RUSSELL expressed it in his address on " The Changing Outlook in Agriculture " at the Centenary Meeting of the British Association in 1931 : " It is difficult to overrate the value of the service he has rendered to South Africa as a whole and to farm animals the world over. He began at the time of the rinderpest plague, a virus disease which killed almost the entire cattle population of South Africa ; the country was devastated by horse-sickness, blue tongue of sheep, heartwater of cattle, sheep and goats, and other terrible diseases. With almost uncanny precision he diagnosed the causes and discovered curative measures ; he founded the Veterinary Research Laboratories at Onderstepoort, of which not only South Africa but the whole Empire is proud, and he trained up a body of veterinary research workers who are extending the good work."

He was an indefatigable research worker and a great teacher. His publications number over 250 and cover a range so wide that many who recognised his eminence in one field were hardly cognisant of it in another. Diseases caused by bacteria, by poisonous plants, by protozoal parasites, by nematodes, by viruses, and by nutritional deficiencies, all came within the sphere of his investigations.

THEILER was honoured by every country in the world. He received seven honorary degrees from universities in three continents, was elected honorary member or fellow of twenty-three scientific societies, received a knighthood from GEORGE V and a royal decoration from Belgium. He was the first recipient of the Medal of the South African Association for Advancement of Science, and of the Laveran Medal from France, the second recipient of the Budapest Prize from the International Veterinary Congress and of the Medal of the Royal Agricultural Society of England. Amongst his later honours was his election to the French Academy of Sciences in 1933.

Born at Frick in the canton of Aargau he received his early education at the gymnasium of Aarau and the universities of Zürich and Berne, taking his professional qualification in 1899. In 1893 he married Emma Jegge, a friend of his school days. Two sons and two daughters were born of that marriage, all of whom, with Lady Theiler herself, survive him.

At his known wish he was cremated. His ashes will rest at the Institute he created at Onderstepoort, in the land in which he carved his career.

HENRY H. GREEN.

TRANSACTIONS

OF THE

ROYAL SOCIETY OF TROPICAL MEDICINE

AND HYGIENE.

Proceedings of an **Ordinary Meeting** of the Society, held at
Manson House, 26, Portland Place, London, W.1, at 8.15 p.m.,
on Thursday, 21st January, 1937.
SIR ARTHUR BAGSHAWE, *C.M.G.*, M.B., D.P.H., *President*, in the Chair.

The President : Ladies and gentlemen, this is the occasion of the first
Chadwick Lecture of the Society, and we have been most fortunate in securing
an American colleague, Professor R. P. STRONG, of Harvard University, U.S.A.,
to deliver it.

This Society has already recognized the eminence of Professor STRONG in
tropical medicine by electing him an Honorary Fellow ; but, in case there are
any present who are unfamiliar with his record and achievements, I propose to
give you a short account of his work in the field of tropical medicine.

RICHARD PEARSON STRONG, after graduating at Yale University and pursuing
his medical education at the Johns Hopkins Medical School, received his M.D.
degree in 1897. In the following year he entered the United States Army, and
a year later went to Manila, where the Spanish Colonial Government had just
been succeeded by a military Government under the United States. Dr. STRONG
became Chief of the Biological Laboratory of the Philippines Bureau of Science,
a post he held for some 13 years. When he arrived in Manila he found that
dysentery was the most serious and common disease, causing, as it did, four
times as many deaths in the United States Army there as any other single
disease. Dr. STRONG and his colleagues found that in the Philippines there were
three distinct forms of dysentery : amoebic, bacillary, and catarrhal. The
" bacillus of Strong " is familiar to us in the literature of bacillary dysentery.

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By his differentiation of these dysenteries Dr. STRONG opened the way to their proper treatment, while his investigations into their method of spread by means of infected drinking water enabled preventive measures to be taken.

Bubonic plague also was rife in Manila, and Dr. STRONG organized a service for the bacteriological examination of rats, and for the vaccination of people living in infected areas whence the rats were collected. In that way he was able to free Manila of this scourge. He rendered a similar service to the Philippines in regard to cholera, a disease which first appeared there in 1902, and in 1905 gave rise to a serious epidemic. Here, again, by his work on bacteriological diagnosis and on the preparation and standardization of cholera prophylactics, he did yeoman service in controlling the disease.

His experience of plague in Manila led to his going, in the winter of 1910-11, to Manchuria, which at that time was ravaged by pneumonic plague. Here again his investigations increased our knowledge of this disease.

In 1907, Dr. STRONG was made Professor of Tropical Medicine in the University of the Philippines, and in 1913 he returned to the United States to take up the post of Professor of Tropical Medicine at the Harvard University Medical School, a position which he still holds. As Professor there he became the leader of several expeditions for the study of tropical medicine in various parts of the world. In 1913 his expedition to South America led to the valuable studies of oroya fever and verruga peruviana. In 1924-5 the Harvard Expedition to the Amazon, led by STRONG, produced further studies of like value. There followed the Harvard Expedition to Liberia and the Belgian Congo in 1926-27. No doubt we shall hear something about that expedition to-night. Leprosy, trypanosomiasis, beriberi and other diseases have been studied by STRONG. During the War period STRONG was in charge of important investigations in Europe on the subjects of trench fever in France and typhus fever in Serbia, while immediately after the War he became General Medical Director of the League of Red Cross Societies at Geneva, where his work laid the foundation of the League of Nations Health Organization, as we now know it.

From this somewhat inadequate account of Dr. STRONG's work you will realize how wide a field it has covered and how he has helped to build up our knowledge of tropical diseases in many parts of the world.

I have now great pleasure in calling upon Professor STRONG to deliver his lecture.

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE CHADWICK LECTURE.

ONCHOCERCIASIS IN CENTRAL AMERICA AND AFRICA.

BY

R. P. STRONG, C.B., PH.B., M.D., Sc.D.,

Professor of Tropical Medicine, Harvard University, U.S.A.

I appreciate very greatly the honour you have done me and the University I represent by inviting me to deliver the first Royal Society of Tropical Medicine and Hygiene Chadwick Lecture in Manson House. I feel very forcibly as I stand here this evening the great debt of gratitude that we in the United States—and, indeed, the people of the whole civilized world—owe to Sir PATRICK MANSON and other scientific investigators in Great Britain, such as Sir DAVID BRUCE, Sir RONALD ROSS, Sir WILLIAM LEISHMAN, and many others greatly distinguished in the field of tropical medicine, who have done so much for the advancement of our scientific knowledge, so much for the alleviation of suffering, and for the prevention of tropical disease throughout the world. In 1935, one of your Honorary Secretaries, Dr. FAIRLEY, was kind enough to come and be our principal guest and speaker on his recent researches, at the Annual Meeting of the American Society of Tropical Medicine ; and a few months ago we had the good fortune to have a visit from Dr. and Mrs. ERNEST MUIR, Dr. MUIR speaking on his experiences and studies in India. So that when your invitation came I felt that it was not only a pleasure to accept, but a responsibility that I ought to try to fulfil. For these meetings and exchanges, I think, emphasize the very friendly and cordial relations and the co-operation that exists between the people of your country and my own. In addressing an audience of this nature, in which so many authorities upon tropical diseases are present, I must ask your indulgence if I sometimes refer to facts already familiar to you.

In order to make clearer the nature of onchocerciasis and the conditions under which it is acquired, I shall illustrate my remarks with cinema films and lantern slides.

In Central America, the disease is sometimes termed the " blinding filarial disease," and hence the parasite which causes it was named *Onchocerca caecutiens.*

The most striking clinical manifestation is the occurrence of subcutaneous fibroid nodules or tumours which vary in size from about 2 to 3 mm. to 5 to 6 cm.

in diameter.   However, the location and number of these nodules or tumours vary greatly in different individuals and in different parts of the world.   Thus, from the earlier investigations of FÜLLEBORN, BRUMPT, BERNARD, OUZILLEAU, LEIPER, SHARP, MACFIE and CORSON, CHESTERMAN, MOUCHET, DUBOIS, CLAPIER, RODHAIN, VAN DEN BRANDEN, and others, it had become established in those parts of Africa where onchocerciasis has prevailed for many years that the subcutaneous fibroid nodules or tumours which characterize the disease are in general situated upon the trunk, frequently in the intercostal spaces, or about the pelvis, or sometimes about the joints, and rarely upon the head.   However, BLACKLOCK, who found in Sierra Leone in the majority of cases the nodules upon the trunk, found in one village eight patients who had the nodules on the head.   MAASS and SAUNDERS, RODHAIN and others also observed isolated cases in Africa with nodules on the head.

In the literature regarding the disease in Africa, prior to 1932, one found no definite evidence that ocular disturbances are connected with the onchocercal infection.   On the other hand, in Guatemala, the fibrous nodules or tumours are generally situated in the region of the head or scalp, and from the time of the first description of the malady in that country by ROBLES in 1915, the disease has been associated in a certain percentage of the patients with ocular disturbances and blindness.   This has also been the case regarding the disease in southern Mexico.

After the demonstration of microfilariae in lesions of the eyes in both Mexico and in Guatemala, HISSETTE called attention to the fact that he had observed onchocerciasis in the north-western Katanga Province in the Belgian Congo, where in many individuals nodules were situated upon the head as well as the trunk, and that in this region the disease was also associated in a high percentage of the cases with blindness.

For this reason I decided to organize an expedition to continue the study of the disease in this locality.   The other members of the expedition were Dr. J. C. BEQUAERT, entomologist ; Dr. J. H. SANDGROUND, helminthologist ; Mr. B. L. BENNETT, technician ; Mr. HENRY MALLINCKRODT, photographer ; and Mr. R. STUYVESANT PIERREPONT, Jr., assistant zoologist.   Through the courtesy of the Belgian Government, it was arranged that Dr. HISSETTE should accompany us to the Province of Lusambo as oculist, and in this locality he carried on with us investigations upon the disease.

After official arrangements regarding the expedition had been completed with the Belgian Government in Brussels, we sailed from Antwerp and disembarked, after a voyage of 16 days, on the west coast of Africa at Lobito Bay, then proceeded by rail for 4 days eastward to the city of Elisabethville, and subsequently, also by rail, north-westerly for $2\frac{3}{4}$ days to the village of Luputa. From here we travelled by automobiles and trucks northward to the village of Kabinda.   After pursuing studies here we proceeded further northward to the village of Pania Mutombo on the Sankuru Lubilash River.   This river we

ascended in a whale-boat to the village of Mulubuli. Here we disembarked and later proceeded on foot by trails (safari) to the districts most severely infected with onchocerciasis, situated between about 5 to 6° south latitude and 23 to 24° east longitude. While in this section of the country, our headquarters were established particularly in the villages of Kassende or Malela. Although the disease was found to be much more prevalent in these regions, nevertheless, cases of onchocerciasis were encountered along our route at all the villages at which we stopped north of Kabinda.

From this itinerary it should be evident that the most seriously infected districts are isolated at considerable distances from civilization. We found no white people residing in this area, and there were no missionary stations there. The village of Kassende, the residence of the Chief of that name, is one of the largest and most centrally situated of the district, with a population of some twelve hundred people. It is surrounded at distances of a few kilometres by a number of other similar villages, and, being the residence of the Chief, it is especially visited from time to time by the surrounding inhabitants.

The inhabitants of the region are Bantus, mainly of the Basonge tribe, though in some sections Balubas and in others Babingis are frequently seen. To the westward, on the edge of the forest, pygmies, especially Batwas, were encountered. Extensive infection with onchocerciasis was also found among the pygmies.

The Basonge people are among the more industrious of the African tribes. They are agriculturists. They also do much weaving, chiefly of the piassave palm (*Raphia vinifera*), for clothing and mats. They travel long distances to trade with the surrounding people. In addition to raising produce for their own food, particularly mandioca and maize, they are especially engaged in the cultivation of cotton. Their agricultural pursuits are of importance in connection with the prevalence of the disease.

On account of the intensity of the sun's rays, particularly between 10 a.m. and 4 p.m., during the dry season we did not attempt to use tents, but lived in the native huts with mud walls and grass roofs. When recently constructed, these are clean, hygienic and comfortable. We established our laboratories and clinics under the sloping roofs of the larger huts where several hundred patients congregated daily in the open, many with marked disturbances of vision, or who were almost completely blind. A special study of the eyes was made in this and other villages, 156 cases, all with ocular disturbances ; and tissues of the eye of 40 of these have been studied histologically.

The experiences encountered in this region, isolated from civilization, were entirely unique and perhaps would not be encountered in any other part of the world. First there was the discovery that practically the entire population of the region was infected, since every individual whom we carefully examined was found to harbour the parasite, and we examined all but very few.

There was a daily visitation at our clinic of several hundred of these people

seeking assistance and relief, many of whom had to be led from the surrounding villages because of failing eyesight or because they were already blind. This large number of patients, closely huddled together upon the ground, sitting in the open, usually with no shelter from the rays of the sun, would patiently await their turn for treatment, often throughout the entire day, and only return to their homes as darkness approached.

From a clinical standpoint the association of ocular disturbances with the disease is emphasized by the high percentage of failing vision and blindness in a locality where some 95 per cent. of the population are infected with the parasite and have demonstrable nodules. Reports of the prevalence of ocular complications in onchocerciasis vary considerably in different regions. In south-western Mexico, LARUMBE (1926) reported that in Chiapas, of 4,000 cases of onchocerciasis, about 800 had developed keratitis, iritis or choroiditis, and 100 were totally blind. HOFFMANN (1930), in the report of his studies also made at Chiapas, does not give the percentage of onchocerciasis with disturbances of the eyes, but says that it is evidently high. However, MÜHLENS (1932) writes that at La Granja, Chiapas, about 10 to 20 per cent. had ocular disturbances. FÜLLEBORN (1930) found ocular complications relatively seldom among the cases of onchocerciasis he saw in Guatemala. No studies of the eye tissues were made. In Guatemala, among our *Onchocerca* cases, disturbances of the eyes were encountered in only about 5 per cent. However, MIRA and DIAZ (1935) report that in the examination of 742 people in six coffee plantations in Guatemala 34·8 per cent. complained of photophobia and 15 per cent. suffered from amblyopia. HISSETTE, in the north-west Congo, in 1932, found the number of cases of eye disturbance very high. In the village of Illebo, of 150 persons, sixty-eight had disturbances of the eye due to the onchocercal infection; fifteen (10 per cent. of the population) were blind; 42 of the 68 with disturbances of the eye had nodules upon the head.

As has been emphasized above, prior to 1931 the association of ocular disturbances with the disease in Africa had not been demonstrated. However, in a very high percentage of the cases observed the tumours had been encountered upon the trunk or extremities and not situated upon the head. Experience in Africa and Guatemala seems to indicate that those individuals in which the nodules or tumours are located upon the head or shoulders are more likely to suffer with disturbances of the eyes; and that in cases in which the tumour is located at considerable distances from the head ocular lesions are usually not present. It has been conclusively demonstrated that the microfilariae are found in greatest number in the skin in the vicinity of the tumours, and that at considerable distances from the tumours very few or no microfilariae may be encountered. In Guatemala we found the microfilariae in the skin most abundant in that of the face; usually very few or none in that over the ankles or feet. Whether or not the microfilariae are positively phototropic, it seems obvious that the tissues of the eye are localities in which they are especially encountered, and

the microfilariae are much more numerous generally in the ocular conjunctivae in cases with nodules about the shoulders and head than in cases with nodules elsewhere on the body.

Recently in Africa, D'HOOGHE (1935), in the examination of 3,448 natives, found that 2·1 per cent. presented ocular complications and that 0·4 to 0·5 per cent. had become blind. However, in the very great majority of the cases nodules were situated upon the trunk, the number of fibromata upon the scalp being only 5·7 per cent.

BRYANT (1935), who has recently made a study of " Sudan blindness " in the Anglo-Egyptian Sudan, believes the condition to be due to *O. volvulus*. Clinically the condition gave the ophthalmoscopic appearance of a diffuse retino-choroiditis, with optic atrophy, often with sclerosis of the retinal vessels and the deposition of masses of pigment on the retina. He remarks that perivascular reaction, so characteristic of onchocercal keratitis, was not present, and that the cornea was not vascularized as in keratitis, but that there was considerable vascularization of the retina.

Obviously it would be wrong to conclude, because microfilariae are merely present in small number in the bulbar conjunctiva, that the disturbances of the eye are necessarily due to the filarial infection. It should be emphasized that in many cases of onchocerciasis the microfilariae in the skin may not produce any disturbance of moment. So also in the eye, the mere presence of a small number of microfilariae in the bulbar conjunctiva may not give rise to any lesions of the eye that are demonstrable. In many tropical countries disturbances of the eyes are common for various reasons, the individuals being predisposed to such affections through their low degree of intelligence, their poor knowledge of hygienic conditions, and their general mode of life. In tropical countries, heat, wind, dust, and smoke within the house or hut are all important in bringing about ocular disturbances. Avitaminosis, xerophthalmia, especially when associated with bacterial infection, trypanosomiasis, and arsenical poisoning may also be responsible for much blindness in regions where onchocerciasis prevails.

It is of some interest also to summarize and compare other conditions under which the disease prevails in Lusambo with those in other African localities and in Guatemala and Mexico. It is especially through ecological studies that our knowledge of the disease and its control has been advanced. Thus it has been shown that the prevalence and spread of the infection are especially dependent upon the geographical, climatic, and botanical conditions which are favourable for the breeding and life of the fly which transmits the infection. The agricultural pursuits and habits and customs of the inhabitants are evidently also of importance in connection with the prevalence and dissemination of the infection. In the endemic districts in Guatemala the terrain in general consists of innumerable ridges separated by deep valleys, with more or less steep slopes and at the bottom of each valley there are one or several more or less swiftly-flowing mountain streams in all of which *Simulium* flies breed abundantly.

The grassy, well-watered villages about which the coffee plantations have developed are situated on the slopes of the rounded hills about these valleys.

In Guatemala and southern Mexico the disease is only found upon the Pacific or southern slopes of the volcanic ranges, at altitudes between about 2,000 and 4,500 feet, and it is especially connected with the coffee production, the best coffee being produced in these regions and at these altitudes. It is in connection with the production of coffee that the inhabitants are especially brought into contact with the fly that transmits the disease. In Guatemala, in the different coffee plantations in which we worked, from 40 to 66 per cent. of the inhabitants are infected.

Onchocerciasis does not exist endemically in Guatemala and Mexico at altitudes below 2,000 feet, because the species of *Simulium* fly which transmits the disease there, *S. avidum* (syn. *metallicum*), *S. ochraceum* and *S. mooseri*, do not breed below that altitude.

Human beings infected with the disease constitute the most important focus of infection. This is emphasized by the fact that onchocerciasis is not found in certain coffee-producing districts in which the altitude and climatic conditions correspond to those of districts in which the disease is endemic, and in which these three species of *Simulium* abound and the inhabitants are similar.

In the province of Lusambo, in Africa, where onchocerciasis is prevalent to an even greater extent than in Guatemala, one finds the terrain characterized also by ridges and hills separated by volcanic crevices and rather narrow valleys, with steep banks and running streams at the bottom, these being surrounded by areas of dense tropical vegetation or forest. In these streams *Simulium* is also found breeding extensively. Only in the vicinity of the villages and plantations has the vegetation been extensively cleared. However, while in Guatemala the coffee production is the agricultural pursuit which especially predisposes the inhabitants to infection, in Lusambo there is practically no coffee production. Here, the production of cotton and of mandioca and the collection of the piassava palm are factors which predispose to infection, and it is these agricultural pursuits which especially bring the inhabitants into contact with *Simulium* flies. Another factor which especially brings the inhabitants into contact with the fly in Lusambo is the frequent bathing in the streams, and the almost daily collection of drinking water from the streams, the water being usually carried either in gourds placed upon the head or suspended from poles resting upon the shoulders of two individuals. The natives of Guatemala, probably especially on account of the cooler climate, do not bathe in the streams. Their collection of drinking water is also much simpler. The frequent travel of the natives of Lusambo to other villages, often at a considerable distance, also aids in spreading the infection.

In Africa, *S. damnosum* is the species of black fly which is particularly concerned in transmission of the disease first demonstrated by BLACKLOCK. In Lusambo we found that *S. neavei* is also concerned in its transmission.

Both species of these flies are frequently found breeding at altitudes below 1,000 feet. Corresponding to this distribution of the fly, we find that onchocerciasis prevails very extensively at altitudes at or below 1,500 feet, as in Liberia, Sierra Leone, and parts of the Belgian Congo, along the Congo River, and in Lusambo (Katanga).

There is considerable variation not only in the location of the tumours, but also in their number in different endemic centres of the disease. In Guatemala, Mexico, Sierra Leone, Liberia and parts of the Belgian Congo, the number of tumours generally may be said to vary usually from one or two up to five or six, though more rarely cases with even more nodules have been reported, as, for example, one in the Belgian Congo by RODHAIN with twenty-six nodules, and one by CALDERON in Guatemala with nineteen nodules. In Lusambo we found in a great many individuals numerous small nodules scattered over the head, shoulders and trunk, varying in number from several up to 150 or even more. The explanation for the occurrence of these numerous multiple infections in Lusambo seems clear. In Guatemala we did not find more than 5 per cent. of the wild *Simulium* flies in the endemic districts infected with the parasite, and the rate of human infection in the different villages varied between 40 and 66 per cent. In Kassende, however, we found 33⅓ per cent. of the wild flies infected, and almost every individual residing there that we examined was infected. It seems clear that in Lusambo the high rate of human infection and the large number of people with multiple tumours corresponds and is especially dependent upon the high rate of *Simulium* infection, and that the lower percentage of human infection in Guatemala corresponds to the lower percentage of fly infection in that country. In Lusambo, pruriginous and xerodermatous conditions of skin were also found to be more common than in Guatemala.

In districts in which the disease is sharply circumscribed, as in parts of Guatemala, the eradication of the human foci of infection is most important. However, protection from the bites of *Simulium* and destruction of these flies are most desirable both in Guatemala and especially in other countries where the disease prevails. In Guatemala the eradication of the *Simulium* concerned in transmission in the endemic areas is very difficult, for its breeding places are so widely distributed in practically every flowing stream of water in the neighbourhood, and such streams constitute the only water supply of the districts. Eradication of the flies through attempting to destroy the larvae and pupae in the streams by changing the vegetation along the banks, by cutting and raking weeds on which the larvae or pupae are fixed, and by the removal of logs and stones or the scrubbing of them with stiff brushes, have all been suggested, but these are not very practicable preventive measures. Oiling of streams, particularly with phinotas oil, O'KANE has found, especially in New Hampshire, to offer a certain measure of value for control of *Simulium*. In streams or waterways in which fish of value are not present, the larvae may be rapidly killed, and the problem of concentration of the oil in the water does not offer much difficulty.

Observations made in Guatemala during several years seem to show that the infected individual constitutes a most important focus of infection. Surgical removal of the tumours containing the adult parasites has been in vogue for several years in this country as a public health procedure of importance for the eradication of the disease. Where a systematic attempt has been made in sharply circumscribed areas to eradicate the disease, the rate of infection has been materially lowered. In one plantation, Moca, where the campaign against the disease was rigidly pursued during 1931, upon re-examination of the inhabitants in 1932 the rate of human infection was found to be greatly reduced, as was also the rate of fly infection. However, the latter may have been somewhat influenced by the climatic factors in that area in 1932. During the next 4 years, when the campaign against the disease was relaxed, the rate of infection rose again. In 1935, an active campaign was again undertaken in Guatemala under the direction of Dr. DIAZ. On account of the difficulty in many instances of the surgical removal of the tumours, attempts were made to destroy the adult parasites in them by the injection of a number of different drugs. The accompanying Tables I and II, (of which the figures have been kindly furnished me by Dr. DIAZ, Mr. OWEN SMITH and Dr. OCHOA), show the number of individuals who have been examined and the percentage showing nodules. There are also indicated the different methods of treatment employed in 1935 and the results of this treatment as observed in 1936. The figures apparently indicate that the treatment employed in the sterilization of the tumours by the injection of several drugs has been fairly effective and that the rate of infection in 1936 was distinctly lower than it was in 1935. The rates of infection in these plantations in 1931 and 1936 are also compared in Table II. Only in Moca has the work of eradication been specially prosecuted.

In Africa, where the disease in many localities is not sharply circumscribed, and in those in which the rate of human infection and of fly infection is very high, the eradication of the breeding places of the fly in the endemic regions constitutes the most important problem. Eradication of human infection in such districts would be entirely impracticable without the elimination of the fly. This eradication probably can only be accomplished by extensive sanitary engineering projects, and by the provision of a modern water supply for the district. Such changes as gradually occur in the building of modern residential centres should eventually result in the gradual extermination of the disease. By these and similar measures, the breeding places of the flies will be removed or gradually reduced, and the inhabitants will no longer come into intimate contact with, and will not be exposed to, the bite of such flies.

In connection with the study of the origin of the disease, in Africa, during our recent expedition, investigations were made by the writer (assisted by SANDGROUND and BENNETT) to see if another mammalian host besides man could be found for the parasite *Onchocerca volvulus*. With this object in view, an examination was made of every animal shot. Though game was not plentiful

ONCHOCERCIASIS IN GUATEMALA.

TABLE I.

Number of Towns Visited, 176.			

1935.

24,431 persons examined ⎫
7,459 with tumours ⎭ 30 per cent. infected.

1936 (first 7 months).

11,580 persons examined ⎫
2,205 with tumours ⎭ 19 per cent. still infected.

310 cases of Onchocerciasis, treated 1st July, 1935, re-examined 10th August, 1936.

Tumours.	Injected with Violeta de Genciana.	Injected with Hexylresorcinol.	Total Tumours Injected.
Persisted ...	58 = 26·2 per cent.	38 = 42·7 per cent.	96 = 30·9 per cent.
Smaller ...	71 = 32·1 „	28 = 31·5 „	99 = 31·9 „
Disappeared	92 = 41·7 „	23 = 25·8 „	115 = 37·2 „
	221	89	310

TABLE II.

	Number of Persons Examined.	Number of Persons with Tumours, 1936.		Percentage with Tumours, 1931.
Moca ...	1,279	295	= 22 per cent.	40
Santa Adelaida	123	58	= 47 „	58·6
Pacayal ...	634	191	= 30 „	—*
Pacayalito ...	141	56	= 40 „	—*
Santa Emilia...	210	120	= 52 „	54

* No figures available.

in the region where the studies were made, investigations upon small mammals and various species of antelope (reedbuck, roan, harnessed antelope, sable and eland), wart-hog, buffalo and hippopotamus were carried out. A species of *Onchocerca* was found in *Bubalis cafer* and a single specimen in one sable antelope, but this parasite does not give rise to nodules in the buffalo or in this antelope, being found especially in the ligamentum nuchae and resembling, both in this respect and in many of its morphological characteristics, *Onchocerca reticulata* or *O. gutturosa*, which have been found chiefly in the horse and in cattle. However, in eland, in studies carried out by LA RUE and the writer both independently and conjointly, subcutaneous nodules were found

in which a species of *Onchocerca* was present, apparently identical morpho-logically with *O. volvulus.* Sections of those tumours reveal cross-sections of the adult parasites and a similar histological structure to that observed in other nodules caused by *O. volvulus.*

In Northern Rhodesia the cattle show a high rate of onchocercal infection. Here two forms are observed, and these have been studied especially by LA RUE and subsequently by the writer. In one, the cervical and shoulder ligaments are especially involved by the parasite ; in the other form, subcutaneous nodules are present or nodules in the musculature.

Onchocerca nodules have also been found in the intercostal regions of cattle on the Gold Coast, and CAMERON has found that the parasite in these nodules does not differ morphologically from *O. volvulus.* From evidence obtained by LA RUE and the writer in Northern Rhodesia, the infection of *Onchocerca* in cattle seems to be in favour of transmission by *Culicoides* rather than by *Simulium* as STEWARD has shown to be the case with *C. nubeculosus* and *Onchocerca cervicalis* of the horse in England. Recently DAMPF (1936), working in Mexico, has found in the dissection of species of *Culicoides* that they are sometimes infected with larval forms of a species of filaria. Whether *O. cervicalis* of the horse is present in Mexico is not known.

It seems evident, then, that in onchocerciasis, as in the case of sleeping sickness, a species of antelope may sometimes act as a reservoir for the parasite, and that, especially in regions where eland has been domesticated, cattle might acquire infection from these infected antelope. It seems possible also that human infection may even originally have occurred from wild animals, and subsequently from cattle. In a pygmy village where *Simulium* was highly infected, the inhabitants were badly infected with onchocerciasis. Pygmies, as is well known, spend a large part of their lives in hunting wild game ; they do not till the soil or have any regular agricultural pursuits.

As the writer pointed out recently, it may be that in onchocerciasis we have an example of an invasion of man by a parasite which before had been well established within the animal kingdom, and that infection of man may have originally occurred through new contacts with infected animals and insects to which man was not previously or extensively exposed. Whether the origin of the disease in man in Guatemala may also be explained in this manner is not yet clear. Obviously the strains of *Onchocerca* which have now, both in Africa and in Guatemala, become thoroughly established in man are transmitted by *Simulium* entirely independent of other mammals.

Recent reports of the structure of the nodules in onchocerciasis, such as those of MOHAMED ABDEL SHAFI (1931), BAEZ (1935) and SOTO (1936), further emphasize the fact that the histological appearance of the nodules or tumours may vary according to their age and whether the peripheral or central portions are examined, and may be influenced by the number of parasites they contain. The young nodules, which may measure only 2 to 3 mm. in diameter, often

show an inflammatory reaction somewhat granulomatous in character about the adult parasites. Considerable numbers of polymorphonuclear leucocytes and endothelial phagocytes are often found with more numerous small round cells in the neighbourhood of the sections of the parasites. Occasionally plasma cells and eosinophils may be observed. In other tumours the tissues about the cut sections of the parasites sometimes consist chiefly of fibroblasts and a number of endothelial cells lying within a more or less organized fibrinous exudate. However, in none of the nodules I have studied, not even the small ones, was the tissue found to be richly vascularized in the characteristic manner of granuloma, and it has not shown the typical structure of a granuloma such, for example, as is encountered in the lesions of yaws or verruga peruviana. For the tumours in which the inflammatory reaction about the parasite is more acute, SOTO has suggested the name "onchocercoma." In the older tumours outside the areas of mild inflammatory reaction the nodules are composed largely of fibrous connective tissue. In some of these the fibroblasts may be few in number and the fibrogliar fibrils not abundant, the nodules in such instances being composed particularly of collagen fibres forming wavy bundles.

It appears remarkable that, although we have been familiar with human onchocercal infection since 1893 when LEUCKART described the parasite from two tumours removed from African negroes, there has been no report of the study of a case at autopsy in which nodules have been found or the adult parasites discovered. In 1922 MACFIE and CORSON made an important communication reporting three autopsies, one upon a Kru and two on Askaris ; neither nodules nor the adult *Onchocerca* were discovered. However, larval filariae were found in all three cases, but only in sections of the skin. They were not found in the viscera or in the lymphatic glands. In only one case was there slight lichenification. In 1931, in Guatemala I observed a case of onchocercal infection in which there was a nodule on the head and serious loss of vision in both eyes. The patient at the time was suffering severe abdominal pain ; he refused to go to hospital and his family refused permission for us to see him again. He died the following day and was buried. Hearing of his death, I obtained authority from the Government to have the body exhumed. I removed the tumour of the head and the two eyes but was not allowed to proceed further with the autopsy. However, through a small abdominal incision I obtained a piece of the liver. There was fluid exudate in the abdominal cavity and the patient had evidently died of peritonitis. Adult *Onchocerca* were found in the tumour of the head, and microfilariae in fresh and hardened stained sections of the skin and of the different tissues of the eye. No microfilariae were found in fresh or stained sections of the liver.

Recently RODHAIN and GAVRILOV (1935) reported upon the histological study of tissues from a fatal case of leprosy sent them from Léopoldville, Belgian Congo. Neither during life nor during the autopsy was there any report of the discovery of nodules or adult *Onchocerca*. Apparently no blood examination had been made ; no tissues of the skin or eye were sent to RODHAIN for

examination. However, microfilariae were found especially in the mammary gland, in the liver, in small numbers in the spleen, and a few in the kidney. They were also present in the cubital nerve. A few pieces of microfilariae were seen in capillaries of the liver.

In this connection it is well to recall that KLOTZ (1930) has described the pathological changes which may result from the presence of *Microfilaria loa* as observed in the spleen of two African natives. *Mf. loa* was found in the blood of one before death. Histologically, in these spleens there was a diffuse fibrosis becoming denser in some areas. The Malpighian bodies were small and many had apparently disappeared, the lymphocytes being sparse and largely replaced by endothelial cells, with fibrosis along the sinus walls. In other areas the whole splenic architecture was obliterated by nodular masses of fibrosis in which eosinophils were the predominant wandering cells. Many well-stained micro-filariae were encountered in the remnants of the sinuses. About groups of these microfilariae an inflammatory reaction and fibrosis were present. KLOTZ emphasizes that it is evident that the temporary resting of the microfilariae within visceral capillaries is unaccompanied by tissue reaction, while a more permanent abode of them, as seen in the spleen, is associated with inflammation and fibrosis. RODHAIN believes that in the case he reports, he was able to make a diagnosis of onchocerciasis from the study of the pieces of the microfilariae found in the tissues. It is obviously very desirable that complete pathological studies should be made of fatal cases in which the diagnosis of onchocerciasis during life has definitely been made and in which adult onchocerca have been found. Certain observations, and especially those of DYCE SHARP, MACFIE and CORSON, RODHAIN and myself suggest that in man, as in certain of the lower animals, adult onchocerca may sometimes be encountered in the sheaths of tendons (as they occur, for example, in the ligamentum nuchae in cattle) and give rise to no nodular formation.

In conclusion, Mr. PRESIDENT, I must thank you for the very flattering introduction you gave me to-night : you have been very kind to me. I only wish I could feel more worthy of what you have said.

I want also to express to your Honorary Secretaries, Dr. C. M. WENYON and Dr. HAMILTON FAIRLEY, my great appreciation of the many courtesies which have been extended to me in connection with my Lecture since I arrived in London, and I particularly want to thank Miss WENYON, the Secretary of the Society, for all the help she has given me. Both my wife and I are very appreciative of your kindness in giving us an opportunity of meeting the Fellows and the members of their families at the reception after this Lecture.

There are so many authorities on this disease present that I should be very glad to try and answer any questions they may wish to ask. Or if they will bring out any additional points regarding the disease which I have not mentioned, I shall be grateful.

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The literature references to other authors whose work is mentioned in this address will be found in the bibliography at the end of the following article :

STRONG, R. P. (1935). Filariasis. (With bibliography.) *Nelson Loose-Leaf Medicine. Survey of Literature, Service Volume*, pp. 319-397. New York and London : Thomas Nelson & Son.

DISCUSSION.

Professor D. B. Blacklock : I should like to say, first of all, that I think our verdict will be unanimous that Professor STRONG has given us a most interesting account of the whole subject of onchocerciasis.

The only points I would like to raise, now we have been given this opportunity, are in connection with the prevention of onchocerciasis and the plague of *Simulium*. As you are aware, the trouble caused by this fly was very much emphasized long before any human disease transmission was attributed to it. In Europe, around the Danube region, even before it was reported upon in Africa, *Simulium* was considered a serious matter because swarms of the Danube fly caused so much damage to both man and animals.

As regards Africa, from the time of CHRISTY, when THEOBALD first described *S. damnosum*, AUSTEN, WHITE and others reported frequently what a perfect pest this fly was to man and his stock, not only irritating him, but rendering the country uninhabitable where it was prevalent, and causing damage in many ways to the economic condition of the people. In Sierra Leone, when I was conducting my investigation there, it was well known how the natives suffered from the fly. More recently GIBBONS and LOEWENTHAL, and also BRYANT, have also emphasized this. GIBBONS and LOEWENTHAL wrote that in Uganda there existed large tracts of very fertile country which were uninhabited on account of the prevalence of *damnosum*.

I gathered from Professor STRONG's work that in America this aspect of the question has not been so striking. Certainly on the American continent it has been reported that various species of *Simulium* were troublesome to the people ; but looking at the literature, it does not seem to constitute such a pest, in itself, as *S. damnosum* and its congeners do in Africa.

As to the disease onchocerciasis, there seemed at first to be some difference between the American and African disease. In Sierra Leone I paid particular attention to the question of eye lesions, but I was not able definitely to associate any disease of the eyes with the onchocerciasis present in the patients. With regard to possible reasons for the nodules appearing on the head : that was not a common site in my investigations, for I found only one village in which the nodules were on the heads of the patients in any numbers. In that village sixty-six people were examined, thirty-three had nodules, and eight of those

thirty-three had them on the head. On trying to find what the cause of this location on the head could be, the only difference I could find was that the village was greatly overhung with enormous trees, mostly cotton trees ; the road down to the big river close at hand was also overhung so much as to be like a dark alleyway. I suggested therefore that it was possible that in South America the situation of the nodules on the head might be associated with the overhead shade provided for the coffee plants, combined with the fact that in the case of people working in the coffee plantations the body might be better clothed.

With regard to prevention, all sorts of things have been suggested for getting rid of the fly ; personal prophylaxis, including wearing of clothes and Simpsonettes, and even smearing the body with odoriferous substances which are offensive to the fly. Some of these appear likely to be not only offensive to the fly, but repulsive also to man, fish-oil being an ingredient in most of them. Most people felt, however, that these measures would not touch the problem. The best course, it was suggested, would be attacking the breeding places, but the breeding places are not easy to attack. Raising the level of the water of streams, then lowering it again, scraping the channels, impeding the rate of flow in order to asphyxiate the larvae, putting chemicals in the water, and many other measures have been recommended. Further methods to be tried are clearing out the streams in the dry weather and attacking the breeding places when the water is low. HISSETTE advised the clearing of the ground for 500 metres from habitations, and I think that this would have been largely successful in the sort of locality I was working in in Sierra Leone, where the fly hid in the tall grass and did not seem to come out any long distance to attack. If that condition holds throughout the year, the clearing back of the bush from the villages would result in a considerable reduction in the total number of bites.

As regards the prevention of onchocerciasis, removal of the tumours could be carried out in Africa as well as in South America. But Professor STRONG has himself pointed out the difficulty due to the chances of re-infection in the Congo where he has recently found such a high rate of fly-infection.

He mentioned, in one of his books, the question of pressure, and its possible influence in determining the localization of the nodules on the head ; I shall be glad if he can, in his reply, tell us more about that subject. It occurred to me, as it would to any who are familiar with the kind of place in which I was working in West Africa, that if there is one place in the body—except the soles of the feet—where pressure is more persistent and continuous and severe than any other, it is the top of the head, owing to the custom of carrying loads on the head. If pressure were the localizing cause of the nodules one would expect to have seen more head nodules in that part of the world than I did find.

I should like to thank Professor STRONG again for his most interesting and stimulating lecture.

Dr. L. E. Hurtado : While in charge of the Laboratory of the Guatemala City Hospital in 1922, I became interested in the problem of onchocerciasis ;

and while there I had a visit from Professor FÜLLEBORN, of Hamburg, who was also engaged in this work.

During that time I had the opportunity of removing parasites from the nodules of patients who came from the infected areas to the hospital. Moreover, with pepsin and hydrochloric acid (37° in the incubator) I prepared an antigen from the parasites, but having left the hospital staff I have not been able to complete the report on that work.

I found that cases of onchocerciasis had an eosinophilia of from 25 to 75 per cent. and I have used this feature as an aid to diagnosis. In one patient I suspected the disease on account of an eosinophilia of 25 to 35 per cent. ; but he was a native, unable to explain himself well in Spanish, and I could not determine if he really had contracted the infection. Eventually a single filarial nodule was discovered in his groin.

Another case was that of a Director of the University who used to pay frequent business visits to property he owned in the infected district. In 1926 he told me that he could not see very well and that he had detected a little nodule on his scalp. The nodule was removed and he afterwards recovered his vision.

Prof. J. Rodhain (*in absentia*) : Since the publication* of the first case of deep localization of *Microfilaria volvulus* I have had the opportunity of examining various tissues removed at postmortem examination from two natives of the Congo infected with *Onchocerca volvulus*.

As in one of these cases I was able to discover filarial embryoes in various tissues outside the skin, a brief description of the results of my investigations may be of interest.

Case 1.—Bakambamba, native woman, about 30 years old, died at the Tshikapa Hospital, Kasai from pulmonary abscess and confluent broncho-pneumonia. The skin was thin and atrophied. Over the right costal region were three filarial nodules the size of a hazel-nut, hard, rounded, non-adherent to the skin.

Thanks to the kindness of Dr. LASMAN, of the Forminière, who had had the observations on this patient carried out very carefully, and thanks to the protocol of the postmortem examination that he made, I have been able to examine the following tissues from this case : portions of the skin, the lungs, the heart, the spleen, the liver, the left kidney, the left fascia lata, a rib, the patellar ligament especially that of the left side, the wall of the uterus, and the trachea.

In none of these organs or tissues, outside the skin, did I find microfilariae. In the skin itself, however, the filarial embryoes were found to be few in number. Likewise in a fragment cut from the top of the filarial nodules, the embryoes were very scarce. We did not see any in a slice of skin removed from the back of the neck.

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*J. RODHAIN & W. GAVRILOV. (1935). Un cas de localization profonde de *Microfilaria volvulus*. Ann. Soc. belge Méd. trop., xv (4), 551.

The condition seen in this case is, therefore, the result of a filarial infestation of very low intensity, and, after examining the sections of the skin, we were not surprised at our failure to find microfilariae in the deep organs and tissues.

Case 2.—A very old native woman who had died at Lusambo Hospital.

We have no details of this woman's illness, but Dr. HISSETTE, who sent us the organs procured at the postmortem examination, assured us that this was a case of onchocerciasis. This was fully confirmed by our examination. Furthermore, the histological examination of one lung revealed the existence of areas of bronchitis and bronchopneumonia which were probably the cause of death.

From this case we were able to examine various pieces of the skin, the mammary gland, the liver, the spleen, the kidneys, the lungs, the brain, the medulla, the spinal cord, the bone marrow, the meninges, the mesentery, the aponeurosis of the muscles of the back and the large intestine.

In decapsulating the kidneys with a view to cutting out a portion for histological examination we collected in the capsule a dozen adults of *Acanthocheilonema (Dipetalonema) perstans*.

The examination of sections, prepared by freezing or embedding in paraffin, revealed the presence of microfilariae which, in most cases, it has been possible to identify as either *Onchocerca volvulus* or *Acanthocheilonema perstans*.

Embryoes of *Mf. volvulus* alone were encountered in (1) all the pieces of skin, of which two came from the front of the thorax and two from the lower lumbar region of the back ; (2) the connective tissue between the galactophorous ducts of the mammary gland ; (3) the connective tissue of the portal system of the liver—the connective tissue showed the slight hyperplasia to be expected from the age of the deceased ; (4) the aponeuroses and the less dense adjoining connective tissue of the muscles of the lumbar region ; (5) the connective tissue of the mesentery.

Embryoes of *Mf. perstans* alone were found in the capillaries of the choroid plexus.

Finally, *Mf. perstans* and *Mf. volvulus* were found together in the capillaries of the hepatic parenchyma.

Very scanty fragments of sheathless microfilariae encountered in the pulmonary pleura and in the meninges of the brain could not be identified with certainty. Their breadth alone would appear to justify identification as *Mf. volvulus*. As regards the intensity of infection of *Mf. volvulus*, the embryoes were numerous in the skin and in the mammary gland but were scanty in the mesentery and in the aponeuroses. They appeared to be relatively more numerous in the capillaries of the liver than in the connective tissue of the portal system where they were scanty.

As regards *Mf. perstans*, they were found easily in the hepatic capillaries and they did not appear to be rare in those of the choroid plexus.

Outside the skin, of which the dermis showed the well-known cellular infiltration of onchocerciasis and some degree of atrophy of the papillae, there were found no tissue lesions which could be attributed to the larvae of *Mf. volvulus*.

This second case of deep localization of *Mf. volvulus* does not therefore afford any proof of the pathogenic action of these embryoes on the internal organs. It shows only that in man, in the case of an intense infection with *O. volvulus*, the microfilariae invading the internal organs preserve their predilection for the lymphatic spaces of the connective tissues. Their presence in the capillaries of the liver merits emphasis because this was also a feature of our first case. When an infection is not very intense, the dermotropism of the embryoes appears to localize them exclusively in the skin. One may ask if the connective tissue of the eye attracts them to the same extent as does the cutaneous chorion, or do they invade this tissue only when the infection reaches a high degree of severity? This is a question which it does not appear possible to answer to-day.

Dr. G. Carmichael Low : It is with the greatest pleasure that I rise to propose a vote of thanks to my old friend Professor STRONG for coming from America to lecture to us to-night. I have now known Professor STRONG for many years. I met him first in the old days in 1903, in the days of MANSON, at the London School of Tropical Medicine at the Royal Albert Docks, and there began an association and friendship with him which has lasted to the present day.

As Sir ARTHUR BAGSHAWE has told you, Professor STRONG first went out to Manila in 1899, as President of the Board of Investigation of Tropical Diseases. During his 13 years in the Philippines he was Director of the Government Laboratory in Manila, and was one of the Editors of the *Philippine Journal of Science*. While there he devoted himself to the study of the diseases of those islands, particularly diseases of the intestines, including balantidial dysentery, on which subject he published a number of important papers. His name became known throughout the tropical world as that of a keen and enthusiastic investigator.

In 1911 he went to Manchuria for the War Department at Washington and the American Red Cross. With TEAGUE he carried out experiments on pneumonic plague, and showed that the disease was spread by droplet infection, against which the only safeguard was the wearing of the " Mukden mask." In the following year STRONG and CROWELL published an account of experiments on the prevention of beriberi in Bilibid Prison in Manila ; these confirmed the work of FRASER and STANTON, and showed that the preventive agent occurred in red rice, rice polishings, and, to some extent, in alcoholic extracts of these.

In 1913 he was back again in the United States, as Professor of Tropical Medicine at Harvard University, a chair which he has ably filled ever since.

In 1917 he came to France with the American Expeditionary Force, doing duty with the American, British and French Armies as Colonel-in-Charge of the Inter-Allied Sanitary Commission. At the close of the War he received from our King the "C.B."—Companion of the Most Honorable Order of the Bath—and was made an Officer of the Legion of Honour by the French.

Back again at Harvard for a short spell, in 1924, Professor STRONG headed the Hamilton Rice Scientific Expedition to the Amazon. This was described in

a masterly Report, with which many of us are familiar. In 1926 he set out with a team of seven for Liberia and the Belgian Congo on the Harvard African Expedition, a vivid account of which was published in 1930, with more than 300 illustrations. In later years he went on the Guatemala Expedition for the study of onchocerciasis, and then again went to the Congo. Some account of this work we have been given in the lecture to-night.

The reports of these various expeditions constitute valuable contributions to our knowledge of tropical diseases, and are, moreover, beautifully produced and illustrated, so that, quite apart from their high scientific merit, it is a pleasure to peruse them.

Throughout the whole of his very active career Professor STRONG has exercised a stimulating influence on tropical medicine, particularly in America, where the developments in this subject during the past few years have been largely due to his influence. He has been President of the American Society of Tropical Medicine, and was the first President of the recently constituted American Academy of Tropical Medicine. We are proud to number him amongst our Honorary Fellows.

You will appreciate, therefore, ladies and gentlemen, how greatly privileged we have been to listen to Professor STRONG this evening. The address he has given us has been a most fascinating one, and the remarkable films and lantern slides most instructive. It is, therefore, with the greatest pleasure that I propose a very hearty vote of thanks to him for giving us this lecture.

Dr. C. C. Chesterman : Until comparatively recent years America, aided by most European nations, was chiefly noted for its depredations in Africa. The Dark Continent was depopulated to the extent of a hundred million by the slave trade. The United States was not then a highly industrialized country, and there was not much to export to Africa in return for her slaves, so the ships which had been used to carry them over came back in ballast, weighted with stones and jiggers, and these were dumped on the African coast. And so America paid its debt to Africa in jiggers. It was perhaps with the idea of redressing that wrong that Professor STRONG decided to include in his world-wide expeditions one or two to Africa. I understand that he and his colleagues were so successful in removing large numbers of parasites from the African Continent that there was a scarcity in some lines for some time after his visits, and considerable consternation in their serried ranks—for as we so well know in the case of *O. volvulus* : " Even a worm will turn."

My first introduction to Professor STRONG was on a river cargo steamer, which I joined from a canoe in mid-stream. On climbing to the upper deck I saw a number of white men who were obviously not the usual travellers on river steamers, and one of them was valiantly " packing up his troubles in an old kit-bag." It was Professor STRONG. A few days afterwards he paid us a visit at Yakusu with Professor SHATTOCK and Dr. MAX THEILER. He has stimulated many of us. I have recently heard from my colleague, Dr. RAYMOND HOLMES, that he has discovered a focus of onchocerciasis which is causing a good deal of

blindness. In our area we had been familiar with that disease, without blindness, for many years, but this is something new and its discovery has arisen owing to the stimulating work of Professor STRONG and others.

I was very interested in seeing his beautiful pictures; the atmosphere of this room and the scenery in the pictures took us to-night back to Central Africa.

In being asked to second this vote of thanks I have been greatly honoured. We shall all have a very powerful reason for remembering Professor STRONG and the first Royal Society of Tropical Medicine and Hygiene Chadwick Lecture. I have very much pleasure in seconding the vote of thanks.

His Excellency M. Slavko Grouitch, the Jugo-Slavian Minister: Mr. President, may I be allowed, as I was at that time Secretary-General of the Ministry of Foreign Affairs of Serbia, to say a few words in appreciation of the splendid work which Professor STRONG did in Serbia, with several of his colleagues, during the great typhus fever epidemic? I noticed that in reference to Professor STRONG's work some of his activities have been passed over rather quickly. This applies particularly to the work he did in Serbia which I witnessed myself. I can assure you that it was really a wonderful work. I remember visiting the special train which Professor STRONG had arranged for the purpose of, I think it was called, " de-lousing " the army. That, as you can imagine, was no small undertaking; but it was most successfully carried out. I have seen Professor STRONG take off his coat and set to work like all the others.

I am very pleased to have had this opportunity of adding my tribute, and the tribute of my country, to Professor STRONG. I would add that among the Orders awarded to him there is another which has not been mentioned. I refer to the Serbian Order of Saint Sava, which, I am pleased to say, I had the personal honour of delivering to Professor STRONG twenty years ago.

The vote of thanks to Professor STRONG was carried by acclamation.

Professor R. P. Strong (in reply): The hour is late, Mr. President, but I want to thank Professor BLACKLOCK very much for his remarks.

With regard to the reason for the allocation of the tumours in the sites in which we find them; frankly I do not know, and I think the evidence is incomplete of just why they occur so often in one region of the body rather than in another. With regard to the eight cases he referred to in which the tumours were found on the head, I think the reasons he mentioned are sound. Dr. SOTO, in Mexico, published an article a few months ago in which he says that the tumours are likely to occur on parts of the body which are least disturbed, that is, in localities where there is very little motion.

As to Dr. HURTADO's remarks, I merely want to say we never forget the splendid work which ROBLES did in Guatemala which was followed by that of CALDERON, TUNA and many other investigators, in some of which Dr. HURTADO took part, when Professor FÜLLEBORN was there.

I wish to express my hearty congratulations to my colleague Professor RODHAIN for his success in his recent studies on onchocerciasis and upon his obtaining this additional material for investigation.

In the first of these cases in which a clinical diagnosis of onchocerciasis was made in the Congo, RODHAIN found no microfilariae in the sections of the viscera though they were present in small numbers in the section of the skin. This is in accord with the observations reported by MACFIE and CORSON in Africa, and by myself in Guatemala, and already referred to in this lecture. RODHAIN found in his second case twelve adult *Acanthocheilonema perstans* in removing the capsule of the kidney sent him. In this case microfilariae were found not only in the skin but in the viscera and deeper tissues. In sections of the tissues it is often difficult to make a diagnosis of the genus of the parasite concerned from small pieces of the microfilariae. However, apparently Professor RODHAIN has been able to do this in some instances and to differentiate between *Onchocerca* and *Acanthocheilonema*.

The evidence in this case to the effect that the microfilariae are sometimes present in the viscera is in accord with that obtained by Professor RODHAIN in the study of another case (one of leprosy) published in December, 1935.

May I emphasize again the desirability and importance of complete and careful observations at autopsy of all fatal cases, with preservation and histological study of the tissues. Only from such repeated studies shall we be able to learn definitely the distribution of both the adult and larval forms of these parasites.

With regard to Dr. CARMICHAEL LOW's remarks, as he has said, he is a very old and valued friend, and, naturally, he also has been very kind to me; nevertheless, I am very much touched by his remarks. My great inspiration for tropical medicine, in earlier years, came particularly from the London School of Tropical Medicine and from the men who were at the head of it and who, together with the others I have mentioned, carried on for so many years their investigations with such success.

And I thank Dr. CHESTERMAN for the kind reception he and his wife gave me in Africa and for the help he gave us in our work. I am so glad to see them both safe and well and back in London again.

In reply to His Excellency the Yugo-Slavian Minister, M. GROUITCH, and in thanking him for his remarks, I should remind him that it was largely through his efforts as Secretary of State for Foreign Affairs, and the support given by other Serbian officials, that the campaign against typhus in Serbia in 1915 was so successfully carried out. Without the aid of the Serbian Government this would have been impossible. It was Madame GROUITCH who especially acquainted us in the United States and elsewhere with the distressing conditions in Serbia in 1915, and obtained much needed assistance and relief for the suffering people of that country. We also must not forget the splendid work which your Colonel W. HUNTER performed with the army in the campaign.

Again I thank you all for your kind attention.

COMMUNICATIONS.

ATTENUATION OF THE YELLOW FEVER VIRUS BY GROWTH IN TUMOURS *IN VIVO*.

BY

G. M. FINDLAY

AND

F. O. MacCALLUM,*

Wellcome Bureau of Scientific Research, London.

The reasons why animal viruses produce variants are unknown, though the environmental conditions in which such variants are most likely to occur are now being gradually recognized and have recently been reviewed (FINDLAY, 1936a). Among the environmental conditions which tend to alter the characters of certain viruses is prolonged growth in association with embryonic tissues *in vitro*. RIVERS and WARD (1933), for instance, found that vaccinia virus, grown for 2 years in a medium of chick embryo tissue and Tyrode solution, lost its power to produce a definite reaction in the skin of rabbits, though still capable of giving rise to typical vaccinial pustules in man. The change in the activity of the virus for the rabbit epidermis appeared to be due not entirely,

and perhaps not at all, to a gradual reduction in the amount of virus in successive sets of cultures, but to some alteration in the character of the virus itself. HALLAUER (1935) also found that fowl pest virus when grown in embryonal liver tissue rapidly lost its pathogenicity for fowls, while retaining its antigenic properties as an immunizing agent. In the case of the virus of yellow fever, HAAGEN (1933), showed that the neurotropic strain grown in tissue cultures of chick embryo, for more than one hundred passages, exhibited no loss of neurotropism. On the other hand, LLOYD (1936) and LLOYD, THEILER and RICCI (1936) observed on growth of the ordinary pantropic strain of yellow fever in mouse or chick embryo (FINDLAY, 1936b) a gradual attenuation in pathogenicity as a result of which the virus was no longer virulent for rhesus monkeys.

It is thus seen that only certain viruses change their character as a result of growth in tissue cultures. The virus of lymphocytic chorio-meningitis for instance, after more than eighty passages in cultures of chick embryo and Tyrode solution still retains unimpaired its pathogenicity for mice (FINDLAY and MACCALLUM, 1937).

In order to determine more precisely the conditions which cause certain viruses to become changed as a result of growth in embryonic tissues and, in addition, to try to obtain variant strains of yellow fever virus which might be used for vaccination against yellow fever, an attempt was made, as briefly related elsewhere (FINDLAY, 1936b), to grow the virus of yellow fever in mouse carcinomata in vivo.

THE GROWTH OF VIRUSES IN TUMOURS.

It is now recognised that many viruses will grow readily in tumour tissues in vivo. LEVADITI and NICOLAU (1922) were the first to show that vaccinia virus would survive and multiply in the tissues of a mouse carcinoma, though the virus failed to persist in the tissues of a sarcoma. Somewhat later RIVERS and PEARCE (1925) found that vaccinia virus and virus III not only multiply in a transplantable rabbit tumour of epithelial origin, but are carried along with the tumour through an indefinite number of transplants and, despite an immunity developed by the rabbit host, survive longer in the tumour than when injected into the testicles of normal rabbits.

Since then the affinity of a number of other viruses for tumour tissue has been demonstrated. HALLAUER (1931) found that fowl pest virus would survive in cultures containing mouse carcinoma for a considerable period, whereas the virus promptly died out in the presence of other mouse tissues. LEVADITI and HABER (1936) showed that fowl pest virus injected into a mouse, either actually into the tumour itself or intraperitoneally, localizes and grows in the tumour cells. LEVADITI and SCHOEN (1936a) also found that rabies virus would grow in mouse carcinomata, while the same observers (1936b) were able to demonstrate a growth in vivo of vaccinia, but not of herpes virus, in the tissues of the Shope papilloma.

THE GROWTH OF YELLOW FEVER VIRUS IN TUMOURS.

Preliminary observations were made to determine whether yellow fever virus would survive and multiply *in vivo* in association with the cells of a mouse sarcoma, 37S, and a mouse carcinoma, 63, two tumours that have been propagated for very many years by transplantation of cells in mice. It was found that in the cells of the mouse carcinoma the virus was still present 12 to 14 days after injection of 0·01 c.c. of a 10 per cent. suspension of the virus, but no longer present in the cells of the mouse sarcoma. Attention was next directed to the question whether the yellow fever virus could live continuously in symbiosis with the cells of the mouse carcinoma so that it could be carried over at each transplantation of the tumour into fresh mice, the subinoculations being made at intervals of 10 to 12 days. Although five passages were eventually made in this manner with the neurotropic strain of yellow fever virus, considerable difficulties arose owing to the fact that growth of the virus tended to produce early necrosis in the tumour cells, which thus failed in many cases to grow on transplantation into fresh mice. The following method of keeping the virus alive in tumour cells was finally evolved. Young actively growing tumours, 8 to 10 days after inoculation, were inoculated directly with 0·1 c.c. of a 10 per cent. suspension of the virus-containing material in serum saline. 5 to 6 days later the tumours were removed from the mice, a portion containing growing cells weighed, ground up to form a 10 per cent. suspension in serum saline as before and reinoculated into other actively growing tumours. By this means it was possible to ensure that the virus was constantly in contact with living cells, a condition which is apparently essential for its prolonged maintenance in the mouse.

Three strains of yellow fever virus have thus been successfully propagated in mouse carcinoma 63 for periods varying from 6 to 15 months.

The three strains are :—

(I). Neurotropic yellow fever virus after 400 passages in mouse brains.

(II). Pantropic tissue culture virus after 121 passages in mouse embryo Tyrode medium and subsequently one passage in chick embryo Tyrode medium.

(III). Pantropic monkey virus (French strain) maintained by passage in the rhesus monkey or hedgehog.

I.—*Neurotropic Virus.*

The primary inoculum into tumours consisted of a 10 per cent. suspension of infected mouse brain in serum saline. Fifty passages of this virus were carried out. While the virus in the primary inoculum killed mice with encephalo-myelitis in a dilution of 10^{-8} when inoculated intracerebrally in a dose of 0·03 c.c., the virus of the fiftieth passage killed mice in a dilution of 10^{-10}, when inoculated by the same route. There was thus evidence that the virus had undergone active multiplication during its sojourn in tumour tissues.

On the other hand no evidence was obtained to show that the neurotropic virus had undergone any change in character. Throughout the experiment, part of the inoculated tumour suspension not used for the inoculation of other tumours was injected intracerebrally into mice and the period till death recorded. This period never varied from 4 to 5 days from the first to the fiftieth tumour passage. Similarly two rhesus monkeys inoculated intracerebrally with tumour material from the thirty-fifth and forty-second passages died in from 10 to 14 days with typical encephalitis and an absence of visceral lesions. Two hedgehogs, inoculated subcutaneously with material from the forty-second passage, similarly died in 9 and 11 days with the lesions characteristic of the neurotropic strain of yellow fever virus.

These experiments, therefore, show that cultivation *in vivo* of the neurotropic strain of yellow fever virus, in association with mouse carcinoma, does not modify the characteristics of the virus. The results are similar to those obtained by HAAGEN (1933) as a result of growth of the neurotropic strain in association with chick embryo cells *in vitro*.

II.—*The Pantropic Tissue Culture Virus.*

Sixty passages in tumour tissue were made with this strain. Material from each passage was inoculated intracerebrally in mice and the time from inoculation to death recorded. No shortening of the incubation period of 7 to 8 days was noted.

After twenty, forty and sixty passages, three monkeys were inoculated subcutaneously with 1 c.c. of a 10 per cent. suspension of the virus ; none of these monkeys showed any significant rise of temperature during the following 3 weeks, but all the monkeys were subsequently immune to yellow fever. After the same number of passages, three hedgehogs were similarly inoculated subcutaneously. One of the hedgehogs inoculated after twenty passages died with lesions in the viscera 10 days after injection. The other eight hedgehogs presented no signs of illness.

Cultivation of the tissue culture strain of pantropic virus thus produced no striking change in the character of the virus beyond possibly a slight attenuation of virulence for the hedgehog, an attenuation which has also taken place in the tissue culture virus as a result of prolonged growth in chick embryo and Tyrode solution.

III.—*Pantropic Virus.*

The original inoculum consisted of 0·1 c.c. of a 1 in 10 suspension of infected hedgehog liver, lightly centrifuged and filtered through a Seitz K filter. As with the other two strains of virus, some of the tumour material from each passage was inoculated intracerebrally into a batch of six mice and, as shown in Table I, there was a gradual reduction in the incubation period until about

the fortieth passage the time from inoculation to death became stabilized at from 7 to 8 days. In all sixty-five passages in tumours were made with this virus.

After ten, twenty, forty and sixty passages monkeys were inoculated subcutaneously with the infected tumour material. As seen in Table II, there was a gradual loss of pathogenicity for the rhesus monkeys, while the same was true for hedgehogs.

TABLE I.

THE EFFECT OF GROWTH OF THE PANTROPIC YELLOW FEVER VIRUS IN MOUSE CARCINOMA *in vivo* ON THE NEUROTROPISM OF THE VIRUS AS SHOWN BY THE TIME NECESSARY TO PRODUCE DEATH FROM ENCEPHALITIS IN MICE.

Intratumour passage.	Period in days from inoculation to death with encephalitis in mice.*
10	7, 7, 11, 11, 11, 13
20	8, 9, 9, 11, 11, 12
30	9, 9, 9, 9, 10, 10
40	7, 7, 7, 7, 7, 8
50	7, 7, 7, 8, 8, 8
60	6, 7, 7, 7, 7, 8

* Six mice inoculated intracerebrally with 0·03 c.c. of a 10^{-1} suspension of tumour tissue.

Prolonged cultivation in tumour tissues thus produced a loss of viscerotropic pathogenicity, while at the same time, as shown by intracerebral inoculation of mice, there was no increase in neurotropic activity comparable with that produced by prolonged intracerebral passage in mice.

Titrations of the amount of virus present in the tumour showed that the maximum titre was attained 4 to 6 days after inoculation, when 0·03 c.c. diluted 10^{-10} was found to kill mice on intracerebral inoculation. 10 to 12 days after inoculation, the virus killed in a maximum dilution of only 10^{-3}. By removal of infected tumours, 4 to 6 days after inoculation and filtration through a Seitz disc, a filtrate was thus obtained suitable for immunization against yellow fever, and containing a high concentration of virus. One of the difficulties encountered in using tissue culture virus for purposes of immunization has been the low titre of virus obtained after growth in the chick embryo Tyrode mixture. By the use of attenuated tumour virus this difficulty is overcome, but the risk of introducing some other intercurrent virus is increased. In order to exclude the presence of other viruses neutralization experiments were from time to time

carried out, the tumour tissue being mixed with immune yellow fever serum and inoculated, either directly into the brains of mice, or intraperitoneally, with injection of starch into the brains.

Mice inoculated with these neutral mixtures failed to die of encephalitis,

TABLE II.

THE EFFECT OF INOCULATING RHESUS MONKEYS AND HEDGEHOGS SUBCUTANEOUSLY WITH PANTROPIC YELLOW FEVER VIRUS GROWN IN MOUSE CARCINOMA *in vivo*.

Intratumour passage.	Number of animal.	Result.
	Rhesus monkeys.	
10	1	†6th day. Yellow fever
	2	†7th „ „ „
	3	survived—fever—immune
20	4	†7th day. Yellow fever
	5	survived—fever—immune
	6	„ „ „
40	7	survived—no fever—immune
	8	„ „ „
	9	„ „ „
60	10	„ „ „
	11	„ „ „
	12	„ „ „
	Hedgehogs.	
10	1	†5th day. Yellow fever
	2	†6th „ „ „
	3	†6th „ „ „
20	4	†5th „ „ „
	5	†7th „ „ „
	6	†6th „ „ „
40	7	†4th „ „ „
	8	†6th „ „ „
	9	survived
60	10	„
	11	„
	12	„

† = death.

although 3 to 4 weeks after inoculation many of those injected intracerebrally developed small cerebral tumours, the cells having the typical appearance of those characteristic of carcinoma 63. Thus no evidence was obtained that any virus, other than that of yellow fever, was present in the tumour cells.

Microscopical examination of tumours known to be infected with yellow fever virus did not show any characteristic changes produced by the virus, though as previously noted, the necrotic lesions commonly found in the tumour were more extensive and appeared more rapidly than in control uninoculated tumours. The yellow fever virus would seem to have a special affinity for the cells of the mouse carcinoma, for virus is found localized in the tumour, both after intraperitoneal and subcutaneous inoculation of infected material, at a time when virus is no longer detectable in the blood stream of the mouse.

DISCUSSION.

By the technique here described it has been possible to maintain the virus of yellow fever for more than 15 months in an abnormal environment. While the neurotropic and the tissue culture strains, which have already deviated considerably from the pantropic strain, underwent either no further, or only a slight change in pathogenicity ; the pantropic strain underwent a progressive loss in pathogenicity for monkeys and hedgehogs without at the same time increasing in neurotropism. This *in vivo* change in pathogenicity is, therefore, closely similar to that produced *in vitro* by growth in tissue culture. The reasons why tumour and embryonic cells should possess in common this power of attenuating certain viruses is still unknown. All that can at present be said is that the phenomenon provides a further point of similarity between embryonic and tumour tissues.

CONCLUSIONS.

1. By direct intratumour inoculation it has been possible to maintain and induce multiplication of yellow fever virus *in vivo* in the actively growing cells of a transplantable mouse carcinoma.

2. The neurotropic strain of yellow fever virus was carried on for fifty passages in mouse carcinoma and underwent no change in pathogenicity.

3. A tissue culture strain of yellow fever virus, which had already undergone a considerable loss of pathogenicity for monkeys and hedgehogs, underwent, as a result of sixty passages in mouse carcinoma, a further slight decline in pathogenicity for hedgehogs. There was no increase in neurotropism.

4. A pantropic strain of yellow fever virus, highly pathogenic for rhesus monkeys and hedgehogs, exhibited as a result of sixty-five passages in mouse carcinoma a profound loss of pathogenicity for rhesus monkeys and hedgehogs, without any increase in neurotropism.

5. Yellow fever virus has a special affinity for neoplastic cells, since localization in the cells of the mouse tumour occurs after subcutaneous or intraperitoneal inoculation of the virus.

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BENIGN TUBULAR STRICTURE OF THE RECTUM IN THE AFRICAN.

BY
MAURICE ELLIS,*
Nigeria.

Aetiology.

It was the custom not many years ago to ascribe to syphilis all benign strictures of the rectum, other than those due to trauma. It was rarely found however that the whole gamut of anti-syphilitic treatment had effect on either the stricture or the associated ulceration. Dr. G. M. Gray has described two cases from Nigeria which were cured by anti-syphilitic treatment and they are quoted by Lockhart-Mummery (1934). Charters Symonds (1922) gave a description of the condition usually found in benign tubular stricture of the rectum which is so masterly that I quote it.

" It is that which involves (in the majority of cases) the lower three or four inches of the gut including the anal margin, but in some cases reaches into the pelvic colon. It affects the whole circumference of the bowel : it is characterized by bands and bridles of indurated and fibrotic muscle between which are pockets from the bottom of which fistulous tracks may lead into the vagina or on to the surface around the anus. The extreme narrowing occurs at several points, and is so tight in advanced cases as to prevent digital examination or the passage of a small bougie. One characteristic is the presence of hard polypoid growths ringing the anal margin, a condition not seen to the same extent in any other form of stricture. The history given by the patients is that of discharge from the rectum together with difficulty in defaecation extending over some years. In married women, these symptoms frequently date from the early days of married life."

After discussing the fact that anti-syphilitic remedies are rarely of value, he points out that gonorrhoeal proctitis is more common in women than in men. He ends by saying :

" It will be nearer the truth to speak of this form of rectal stricture as due to local infection from labial or vaginal discharges and as the most frequent disease is gonorrhoea to call it ' gonorrhoeal stricture.' "

HAYES (1931), describing cases of stricture of the rectum in America, the majority in coloured patients, states that by examination of smears of the rectal discharge, he found gonococci in 69 per cent. of cases examined. GABRIEL (1932) states that gonorrhoea is a frequent cause of proctitis and that stricture follows in general. W. E. MILES (1935) makes no mention of gonorrhoea specially and suggests chronic uterine infection as the cause.

A new departure in thought about the aetiology of this condition was marked by STANNUS (1933) in his monograph *A Sixth Venereal Disease*. In this he reviews the light thrown on lymphogranuloma inguinale by Frei's isolation of an antigen. Frei's antigen showed that the condition known as " esthiomene," in which there is elephantiasis of the vulva associated with vaginal and rectal discharges and rectal stricture, is related to lymphogranuloma inguinale. STANNUS suggests the name " poradenitis venerea " for all these related conditions. A large amount of work has since been carried out on this subject, which shows that the disease is very common. (An excellent review of this occurs in the *Medical Annual*, 1936.) GRAY (1936) describes eleven cases of poradenitis, in nine of which Frei's reaction was positive, and outlined the pathological progress of the condition. He states that :

" The condition begins as chronic urethritis, which may be accompanied by intra-urethral ulcer. If the inflammatory condition extends deeply into the tissues, the disease may progress so slowly as to allow gradual formation of scar tissue. In some cases the inflammation extends more steadily and rapidly, ulcerating the urethral meatus and extend-ing into the vagina ; there may be an associated elephantiasis of the vulva. The ulceration may burrow deeply into each side of the rectum, sometime producing recto-vaginal fistula."

The following eleven female cases under my care at Bida, in 1935 and 1936, have been arranged to show the different stages of the pathological process described above.

Case 1.—89/36. Nupe, 36 years, complaining of incontinence of urine and vaginal discharge of many years' duration. On examination, the posterior lip of the urethra was elongated and thickened—urethra dilated, red and granulomatous with pus oozing from its surface. Much pus in vagina. Posterior wall of vagina red, with mucosa thrown into the folds and thickened—vagino-perineal fistulae present. No stricture of rectum.

Case 2.—727/36. Nupe, 26 years. Complaining of incontinence of urine and growth of vulva of several years' duration. On examination, the condition of the urethra and vagina was similar to Case 1. In addition there was elephantiasis of the labia minora. No stricture of rectum.

Case 3.—585/36. Nupe, 26 years. 5 years' history of purulent discharge from the rectum and difficulty in defaecation. Examination showed mouth of urethra very red and patulous. Granulomatous, polypoid masses of epithelium just above posterior lip. Much pus. Remainder of vaginal mucosa rugose. But posteriorly at vaginal os, epithelium appeared to be stripped up forming an overhanging fold—only a thin wall of smooth granulation tissue separates vagina from rectum. Two condylomata at anal orifice—sphincter patulous. One inch above anus is a tight stricture, which will not admit examining finger. After dilation, stricture found to be over 2 inches long. Abundant purulent discharge from pockets in stricture.

Case 4.—439/36. Hausa, 38 years. 3 years' history of discharge from rectum and · difficulty in defaecation, together with pain in micturition. Examination showed thickened reddened epithelium in vagina and around urethra. Tags of mucosa at vaginal orifice with

pockets of pus in between. On posterior wall, the epithelium is raised into fold, below which there is much fibrosis—in middle of fibrosis there is small recto-vaginal fistula—vagino-perineal fistula also present. Condylomatous tags at anus. 1½ inches above anus and above recto-vaginal fistula there is a stricture 2 inches long. Abundant purulent discharge.

Case 5.—640/36. Hausa, 33 years. 15 years' history of discharge from rectum with difficulty in defaecation and thin scybala. Examination showed vagina similar to Case 3. Two large polypoidal masses protruding from anus. Small recto-vaginal fistula just above anal orifice. Above this, a rectal stricture stretching upwards beyond the tip of the examining finger, with many pockets of pus.

Case 6.—670/36. Nupe, 35 years. 4 years' history of purulent discharge from rectum and difficulty in defaecation. Examination showed vagina very tight, narrow and fibrous. Mucosa rugose and reddened—posteriorly condylomatous proliferation of epithelium. ¾ inch above anal orifice a tight rectal stricture was present extending as far as the finger could reach. Rectal discharge + +. Minute recto-vaginal fistula below stricture.

Case 7.—F. Nupe, 30 years. One year's history of purulent discharge and difficulty in defaecation. Examination showed elephantoid condition of vulva spreading up to mons, condition of vagina similar to Case 4, but recto-vaginal fistula larger. Stricture of rectum above fistula, ½ inch long. Purulent discharge.

Case 8.—91/36. Hausa, 36 years. Admitted with large ulcer of leg. After admission complained of rectal discharge. Examination showed rugose condition of vaginal mucosa. A short stricture of rectum was present 1 inch above anal orifice—small recto-vaginal fistula similar to Case 5. Purulent discharge.

Case 9.—295/36. Nupe, 36 years. 15 years' history of pain in and discharge from rectum. Examination showed ulcerative condition in perineum resembling incomplete perineal tear. Vaginal mucosa thickened and rugose, otherwise vagina and vulva normal. Condylomatous projection from anus—½ inch above anus very tight stricture 2 inches long, pockets of pus. No recto-vaginal fistula.

Case 10.—92/35. Beriberi 22 years. 3 years' history of discharge from rectum and vagina, with incontinence of faeces. Examination showed plentiful polypoid growths around vaginal and anal orifices. Vagino-perineal fistula and fistulae in anus present. Much pus in vagina with mucosa red and thickened. Urethra similar to Case 1. Anal sphincter patulous and inefficient. Recto-vaginal fistula just above anus. Purulent discharge + +. The condition did not follow child-birth.

Case 11.—112/36. Nupe, 35 years. 4 years' history of incontinence of faeces with a rectal prolapse on defaecation—rectal discharge. Examination showed multiple fistulae around anus and vagina. Vaginal mucosa thrown into pockets filled with pus. Anal sphincter more or less non-existent—large anal fissure. Recto-vaginal fistula size of threepenny piece (nearly 2 cm.). Thick stricture above this, which served as apex for prolapse—not much pocketing of mucosa in stricture. Purulent discharge from rectum and vagina. Patient was certain condition did not follow child-birth.

These cases I think illustrate very well, the stages described by GRAY. In Case 1 we have urethritis, Case 2 urethritis and elephantiasis with so far no involvement of rectum. In Case 3 we have urethritis, and posteriorly in the vagina, an ulcerating process, which has gone through to the rectum and produced a stricture. In Cases 4 and 5 the picture is similar but a fistula has been produced. Case 6 is a further stage, the fibrosis in the vagina being the end result of the vaginal inflammation. Case 7 shows a more extensive stage, where elephantiasis of the vulva has supervened—typical esthiomene. Cases 8 and 9 show, I think, that the inflammation had extended into the rectum, producing stricture, but leaving little trace in the vagina. In Case 10, the inflammatory process is more rapid and has produced widespread damage to

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the rectum, whilst the inflammation is still present in the vagina. In Case 11, there is almost complete disorganization of everything in the vicinity.

I was unable to obtain Frei's antigen to test these cases, but I think the series of pathological pictures corresponds so well with GRAY's description, that, starting from the typical undoubted case of poradenitis venera in Case 1, Case 11 is equally one of poradenitis, if not so typical. The nine cases of stricture of the rectum correspond to the pathological picture given by CHARTERS SYMONDS (loc. cit.) and to that by HAYES and termed gonorrhoeal stricture. But gonorrhoeal urethritis in the male although inflammatory and producing stricture is never an ulcerative process, and never goes on to produce fistulae. The fistula associated with stricture in the male urethra is always proximal to the stricture and is caused by the necessity for urine to escape. In these rectal strictures, any fistula is always distal to the stricture and judging from Cases 3 and 4 appears to be due to the ulcerative process. In 1922, when CHARTERS SYMONDS wrote, not much work had been done on poradenitis. If, in his closing remarks, he had said that " it will be nearer to the truth to speak of this form of rectal stricture as due to labial and vaginal discharges, now known to be due to poradenitis " instead of inferring only that it is due to gonorrhoea, then the value of his paper remains unchanged. But HAYES found gonococci in 69 per cent. of his cases. I made repeated attempts to find gonococci in several of these cases. In only one instance did I think I was successful. I sent the slide to the Pathological Laboratory, Lagos, for confirmation. But Dr. ELMES gave his opinion, confirmed by Dr. YOUNG, that what had been taken to be the gonococcus was in fact a small, Gram-negative intracellular diplo-bacillus.

It is possible that HAYES's gonococci were also diplo-bacilli and that in fact his cases were due to poradenitis ?

TREATMENT.

There are two things requiring treatment in these cases (a) The causative condition with its ulceration, (b) the mechanical condition of stricture. The treatment of each is very disappointing. In the review of poradenitis in the Medical Annual(1936) no form of treatment was found to be uniformly successful, even in the hands of its advocate. None of the common drugs used for anti-venereal (as distinct from anti-syphilitic) treatment or for protein shock was found to be of value. The treatment adopted in Cases 3 to 10 above was bi-weekly dilation with Hegar's dilators and twice daily washouts of rectum and vagina with flavine. GABRIEL states that Hegar's dilator No. 23/24 is the largest safe size to use, but unfortunately, I had only No. 17/18 as the largest.

Cases 3, 4, 6, 8, 9, 10, after treatment varying from 3 to 10 weeks (in each case the patient wanted to go out as soon as any improvement was noted) were discharged much improved. Case 4 was seen in the bush 3 months later and complained that her symptoms were returning. Case 6 is still attending as an out-

patient twice a week for dilatation.   Case 10 was taken over after 5 months' treatment from another doctor.   The stricture had not been detected and rectal and vaginal washouts had not produced much improvement.   Bi-weekly dilatation soon gave some improvement : the patient was discharged 10 weeks later with the rectal sphincter competent and the discharge minimal.

Case 5 was treated by dilatation.   After 4 weeks, the stricture had stretched well and therefore the finger was used to increase the dilatation to correspond to Hegar's 25/26.   The patient developed a high temperature next day, and died 4 days later, with signs of retro-rectal cellulitis.

Thus treatment by dilatation is only palliative and, as shown in Case 5, is not without danger.   The condition recurs, as shown in Case 4, and unless the patient will come for regular dilatation, as Case 6, then the final result is a return of the *status quo*.   As most of these patients live many miles from hospital, regular attendance for dilatation seems almost impossible.

LOCKHART-MUMMERY and LLOYD DAVIES (1935) describe an operation for radical cure of rectal stricture, on the lines of Finney's pyloroplasty, but the operation demands a temporary colostomy.   The following two cases represent my experience of the reaction of the African to colostomy.   Whilst in Lagos, working with Dr. G. M. GRAY, a case similar to Case 10 came under my care. As a first step, a colostomy was performed.   The operation appeared to be satisfactory, but the patient was apparently so depressed by an abdominal anus that she died 10 days later.

Case 11, called for radical treatment and, remembering the Lagos case, a complete perineorrhaphy was tried.   The wound was completely broken down in 5 days.   The patient's consent to a temporary colostomy was obtained.   But 2 weeks after it had been opened, she was begging for it to be closed.   She was appeased by promises and the perineorhaphy was again undertaken a week later.   As soon as the patient recovered from the operation, she made appeals again for it to be closed.   A promise to close it a week after the operation was given.   But the day before the promised operation, she had given up hope, covered herself with a blanket, refused all food and died 2 days later.   The perineorrhaphy had healed perfectly.   "The operation was successful, but the patient died."

It will be seen, therefore, that unless utter and complete confidence can be obtained from the patient, so that a temporary colostomy can be performed, the best treatment we can give is by dilatation with as long attendance for treatment as can be obtained.

SUMMARY.

1.   The aetiology of rectal stricture is reviewed.   It is shown that the old theory of syphilis is no longer accepted, and that gonorrhoea has been held responsible.   More recently poradenitis venerea has been put forward as the

cause. Eleven cases are described showing a graded series of pathological pictures from typical poradenitis venerea to a typical rectal stricture, called formerly gonorrhoeal. It is suggested that these cases show that one pathology can be given for all these cases.

2. The treatment is discussed. Eight cases were treated by dilatation and lavage, with a fatal result in one case. In two cases in which a temporary colostomy was tried as a prelude to radical treatment, both patients died, apparently from the horror inspired by the abdominal anus.

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THE CONTROL OF ANOPHELINE BREEDING IN RIVER BEDS.

BY

H. N. WORTH, M.Inst.C.E., M.Am.Soc.C.E., M.Inst.W.E.,*

*Sanitary Engineer, Medical and Sanitary Services, Ceylon,
Colombo, Ceylon.*

INTRODUCTION.

The necessity for the control of anopheline breeding in river beds, by permanent means, has been keenly felt in Ceylon for some considerable time, and during the recent serious malaria epidemic it was shown that one of the most significant factors which contributed to the cause of that calamity was the condition of the river beds.

A series of experiments has been conducted at Badulla, with the object of formulating a practical scheme for the mass reduction of anopheline breeding in river beds. The results of these experiments have thrown some light on the problem and enabled recommendations to be made regarding the mode of treatment suitable for various conditions. Special reference is made to the control of dry weather channels and to the prevention of pooling in sand-bottomed rivers, also to dealing with pools in boulder and rock-bottomed sections.

The methods proposed in connection with channel control in sand-bottomed rivers have not, so far as is known, been previously applied elsewhere as a malaria control measure. The scheme discussed in this paper is however based upon that used in Burma for controlling channels in order to facilitate the transport of teak from the forests, for which purpose it has proved highly successful although inexpensive. The possibilities of applying permanent devices for the control of anopheline breeding in rivers have hitherto received little more than passing reference, possibly due to the belief that nothing of a practical nature could be done, except at a prohibitive cost.

In every malaria scheme of any magnitude so far carried out, sanitary engineering has played an important role. Extensive swamps have been drained, marshes reclaimed and streams and channels controlled by various engineering devices, but the problem of controlling anopheline breeding in river beds still awaits solution. As an engineering proposition, it presents no insurmountable difficulties, the results of the investigations referred to indicate that a solution of the problem is within sight and that a reasonable degree of success is likely to be obtained by the adoption of measures on the lines of those herein described.

The River Factor in Ceylon.

I.—RELATION OF RIVERS TO MALARIA.

The study of the anopheline fauna of Ceylon, carried out by CARTER (1927) during the past 12 years, has shown *Anopheles culicifacies*, to be the chief—if not the only—carrier of malaria in Ceylon, and to be highly susceptible to malaria infection.

This mosquito breeds freely in wells, borrow pits, rock pools, irrigation channels and quarries, and shows a decided partiality for slowly moving bodies of clear, fresh water, exposed to sunlight and free from heavy vegetation, such as occur in certain river beds during the long, dry seasons. Owing to the frequency with which the larvae of *A. culicifacies* have been found in the pools and shallows of river beds—particularly in the wide sand-bottomed rivers—the problem of devising some permanent form of control has been under serious consideration for some time.

It has previously been observed that malaria may become prevalent in the vicinity of rivers, and the influence which rivers may have in causing and maintaining an epidemic has been clearly shown in the case of the serious epidemic which broke out in Ceylon towards the end of 1934.

It is stated in the official reports (BRIERCLIFFE, 1935; and GILL, 1935) that during the first 7 months of that epidemic some 80,000 deaths from malaria occurred and over 1,500,000 persons contracted the disease. The habitations in the vicinity of rivers gave the first indication of the onset of the epidemic and

were more seriously affected, and for a longer period, than were those situated at a favourable distance from them.

The entomological data collected during that time showed that an enormous increase in the propagation of *A. culicifacies* was taking place in the partially dried-up river beds throughout these areas, although practically all other potential breeding places had disappeared owing to the continuance of the drought.

An extensive survey of the watercourses revealed the fact, that whereas innumerable pools existed in the broad sand-bottomed rivers, due to the gradual narrowing of the dry weather channel, the dangerous pooling conditions in many of the tributaries were often attributable to other factors. Numbers of these tributaries were so seriously blocked by fallen trees, logs and masses of vegetation that natural conditions of flow in these channels ceased to exist, with the result that the formation of backwaters and pools became established soon after the subsidence of the rains. It was perfectly evident that attempts to apply permanent measures to these tributaries would be impracticable until the accumulations of obstructions had been removed.

It is not difficult to visualize the effect rivers in this condition must have upon the village population located in the vicinity, particularly when other factors favourable to the malaria carrier are present and enormous increases in their numbers may be expected.

II.—CONTROL OF LOW WATER CHANNELS.

The control of low water channels of rivers in the malarious areas appeared to offer the only possible, practical and permanent solution for the mass reduction of the malaria carrier, and a measure, which at the same time would undoubtedly have a marked influence in reducing the degree of endemicity in such areas.

Many of the rivers and tributaries in Ceylon vary considerably in characteristics and geological features which frequently change from section to section, and it was realised that a careful selection of the mode of treatment and design would be necessary if a reasonable measure of success was to be expected. Moreover, it is always possible for conditions on a section to change, future circumstances therefore need to be gauged with the utmost caution.

From the standpoint of mosquito control, the wide sand-bottomed rivers present the greatest engineering problem; the courses of their dry weather channels are constantly changing with the extension of the dry weather period, with the result that uneven silting takes place along the bed and channel of these rivers. Sand bars and shoals are formed, causing pools and shallows to occur over extensive stretches, with the eventual breaking up of the stream into numerous rivulets if the dry weather period is prolonged.

The lengths of rock-bottomed reaches are usually comparatively short and the chances of evolving practical and economic forms of treatment often appear

remote.   The variable nature of rock formation offers few opportunities for the adoption of any type form of treatment and usually necessitates special and detailed consideration.

Instances of solitary rock barriers are not infrequently met with and in many cases these are the cause of backwater cuts and subsidiary channels, whilst others probably have a beneficial effect in preventing the formation of shallow pools.

Massive rock formations of considerable length, are by no means rare on certain rivers, the rock sometimes extends to the full width of the river and contains numerous water holes.   The water collections in this type are mostly of a definitely permanent character, and present no serious engineering difficulties in regard to the application of permanent control devices.   The initial cost, however, is likely to be somewhat heavy when large areas are involved, but once permanent works have been completed further expenditure is at an end.

The boulder-strewn stream is a very common type, especially of the tributaries in the foot-hills.   Many of these rivers form particularly dangerous breeding pools and are often subject to extensive pooling during the low stages of the river throughout the dry season ; their location and extent is constantly changing with the flow conditions.

III.—EXPERIMENTAL WORK AT BADULLA.

During 1934-35, experimental work was commenced at Badulla with the object of obtaining practical methods of control to meet the variety of problems. Badulla is situated in the hill country of Ceylon (elevation, 2,250 feet) and includes some 450 acres of rice fields and many irrigation channels.   The problem of malaria control here has received considerable attention and has been the subject of special investigation by the Medical Entomologist who, as a result of field research during 1930-32, showed (CARTER, 1934) that whilst *A. culicifacies* occurs in excavations associated with brick and pottery making, the heaviest breeding takes place in the river beds.   In other types of potential breeding places at Badulla, the larvae of this mosquito were found less frequently and in very small numbers.   In his concluding report (unpublished) on the subject, the Entomologist stated, " I consider that every endeavour should be made to eliminate as many pits as possible and that all pools in the river bed or margins and pits, which cannot be filled, should be oiled regularly.   The treatment of the river itself is a larger problem and is one which should receive the attention of the Sanitary Engineer."   The main river at Badulla, the Badulla Oya, practically encircles the town and its length within the malaria control zone is approximately $4\frac{3}{4}$ miles.   There are in addition, two main tributaries, the Rambukpotta Oya and the Kuda Oya, within this zone, representing an additional length of about 2 miles.

The river is about 150 feet in width and for the greater part of its length has a sandy bed, but in the upper reaches heavy boulder and rock formations occur.

FIG. 1.—View of river showing accumulation of vegetation obstructing free flow.
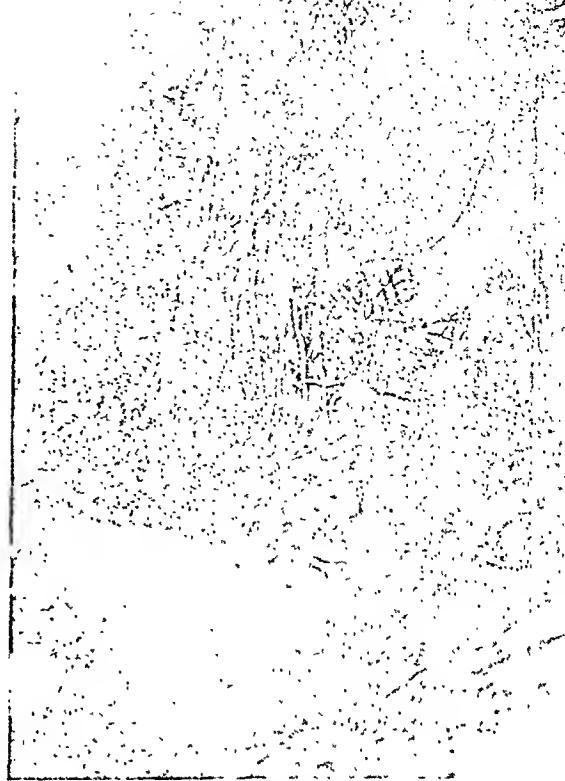
FIG. 2.—Sand bottomed river showing pooling caused by vegetation.
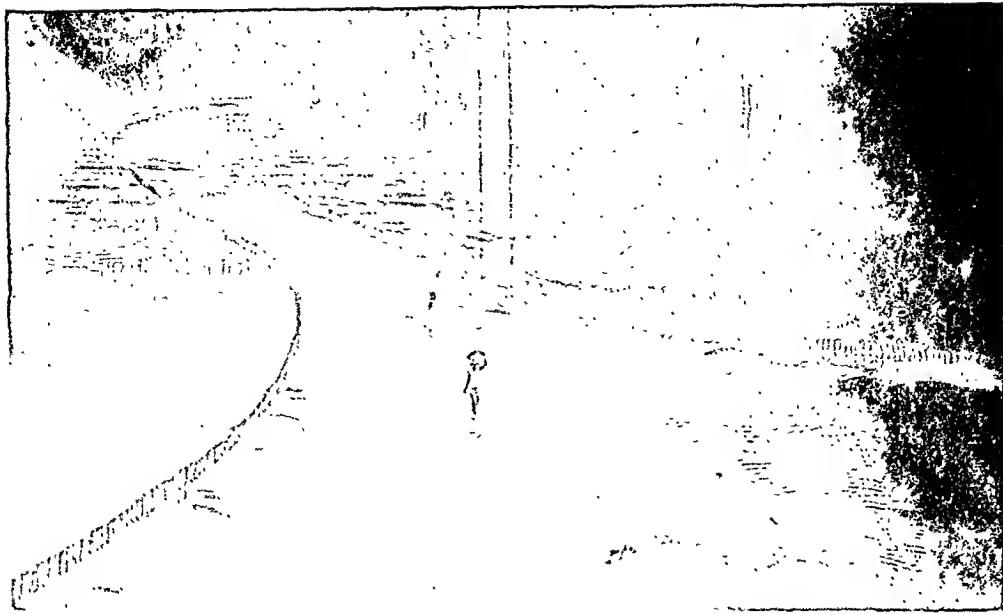
FIG. 5.—Channel control by means of bamboo stakes. (Note silting taking place on both sides of the channel.)
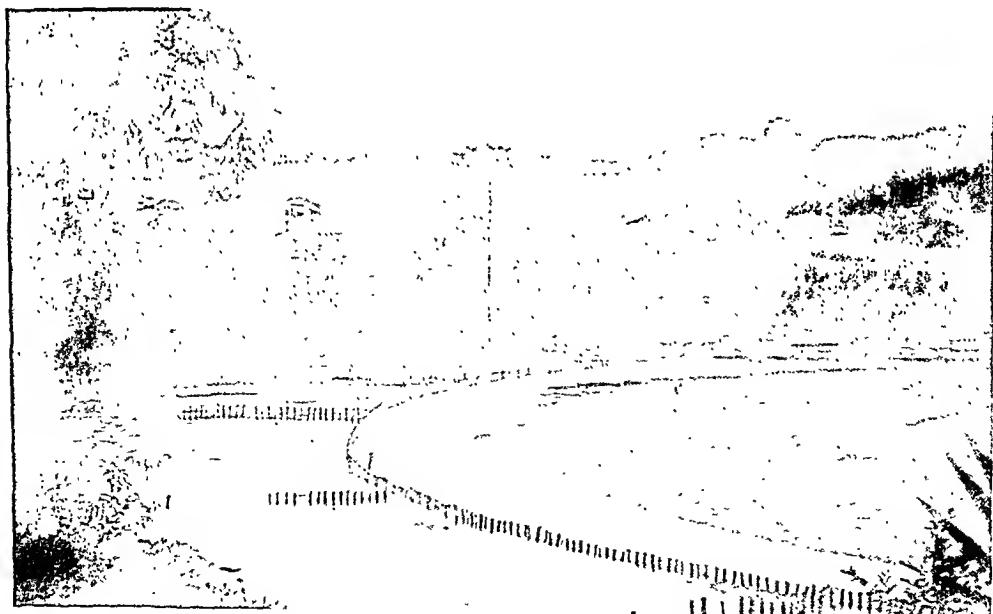
FIG. 6.—Channel control by means of bamboo stakes. (Note gradual silting to left of photograph.)

FIG. 7.—Bamboo boulder filled check dam.

FIG. 8.—Channel control—showing hog wire spur dike in foreground.
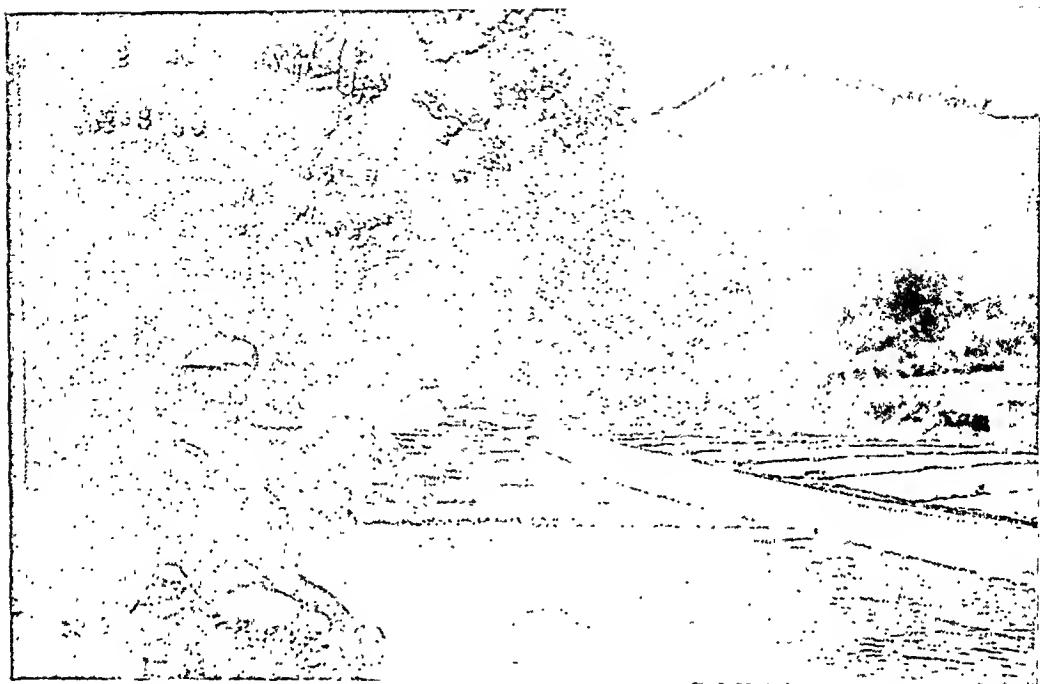
FIG. 9.—Channel control, showing cross dikes.
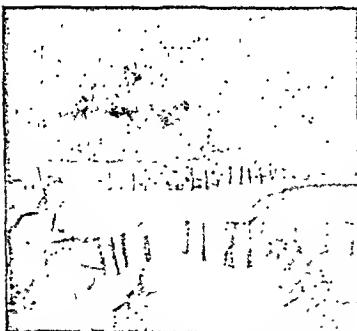
FIG. 10.—Check dam, showing increased flow in channel section.
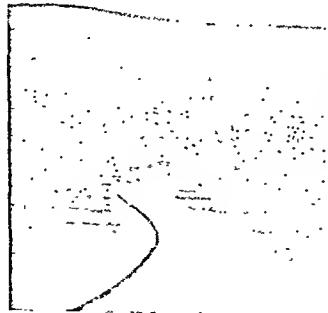
FIG. 11.—General view of completed channel.

FIG. 12.—Drained, filled and sealed pools.

A.—*Prevention of Pooling—Rock and Boulder Section.*

After a preliminary inspection has been made, it was decided to confine the first series of experiments to the upper reaches of the river where numerous pools and bays existed between the rocks and boulders. The procedure followed was that of draining, filling and sealing.

A considerable number of these pools are formed directly or indirectly by obstructions in the river bed, mainly due to large boulders which in course of time become so tightly wedged together that they form series of watertight bays along the edges of the river. In many cases effective drainage was found possible by merely loosening the boulders with the aid of steel bars ; in few instances only was it found necessary to resort to blasting operations.

Where economical drainage was impossible, such as in the case of deep cavities in rocks and pockets between very large boulders, these were tightly packed with small boulders, shingle and gravel up to water level, and finally sealed with 1½ inch cement grout finished with a slight slope stream-wards. Shallow pockets in live rocks were filled with an appropriate packing and sealed in a similar manner.

No difficulty was experienced in ascertaining the correct level of the sealing coat as a clearly defined watermark on the stone indicated all situations where pooling occurred for any length of time. The cutting of chases was occasionally found useful and economical in draining long shallow permanent pools. Where alternative methods were possible, the deciding factor was that of cost. Trials in the sealing of shallow pools with cold asphalt emulsions were made, the finishing layer being a composition containing small grade gravel collected from the bed. This was, however, found to be incapable of withstanding heavy scour but gave fairly satisfactory results in other situations, and was cheaper than cement grout. It is, however, proposed to carry out further experiments, making use of similar materials in combination with a cement key along the edges of the seal.

After the monsoon rains of 1935, the experimental section was inspected and the defective asphalt seals made good. The section is now completed and the results extremely satisfactory. At all stages of the river and under various conditions of rainfall it is now entirely free from shallows and isolated pools.

For the purposes of these tests a most difficult and congested section was purposely selected, but the results have entirely satisfied requirements.

B.—*Prevention of Pooling—Sand-Bottomed Section.*

A second series of experiments was undertaken with a view to overcoming the problem of pooling in sand-bottomed rivers. A detailed survey of a selected section was first made and the low water channel and bed levels were recorded.

The object of the experiments were (a) to ascertain, if the sand in the river

bed could be effectively retained at suitable levels along the sides of the low water channel where deposits already existed; (b) to ascertain if existing deposits in sand spits could be conveniently removed by erosion and dispersed along the toe of the river banks and scoured portions in the lower reaches; (c) to ascertain to what extent bayous, caused by the scouring action of the river, when under flood, could be corrected by the river itself during the fall of the water level.

For the purpose of carrying out these experiments, over 11,000 tetrahedron concrete blocks were cast from metal moulds on the site. The blocks were similar to those used on the Mississippi River revetments in the Memphis area (*Engineering News Record*, 1934). These were laid by hand over existing sand banks which it was desired to retain, also at the inlets and outlets of bayous where silting was desired. The results of the various experiments were observed shortly after the cessation of the monsoon rains and were found to have been extremely effective, both for pinning down the sand and in forming closures to subsidiary channels and bays. In every case it was found that additional sand deposit had occurred, in most cases to a depth of at least 6 inches. Where it was desired to still increase the depth of sand, the blocks were removed and replaced upon the new sand bed. These blocks, which are 12 inches on all sides, were found to be very useful for experimental work and owing to their solidity have a long life enabling them to be used and re-used many times, without expense beyond that incurred in handling. They offer sufficient resistance to the prevention of bed erosion and encourage the gradual accumulation of additional sand where needed. They offer a remarkably small exposed area to the direct force of the flowing water in relation to the area in friction against the bank, and on whichever of the four faces the blocks rest, they are equally effective.

A number of stone filled hog-wire spur dikes or groynes were tried out, with the object of increasing the sand cover along the river edges and for retaining sand on the scoured sections. These were formed by securing together three lengths of woven mesh fencing 30 to 50 feet in length. The mesh was then partially buried in shallow trenches and filled with boulders, the outer edges being brought together and clipped with stout wire. The result was a partially-submerged sausage of stones of about 5 feet in diameter. These were laid at selected points along the bank at an angle of approximately 90° to the dry weather channel. They proved fairly satisfactory, but offered rather too much resistance in times of flood and caused a certain amount of local pooling on their upstream side. The retention of sand between the dikes was, however, satisfactory; they appeared to be particularly suitable for withstanding strong currents and maintaining the course of the channel at sharp bends. They cannot, however, be recommended for use as a malaria control measure until the liability to scour is overcome, either by altering the type or by providing a checking device above them, particularly where they are subjected to high velocities.

C.—Channel Control.

Experimental work was continued during 1936, and consisted of a number of tests in connection with dry weather channel control and the equalization of sand deposit in river beds.

From observations made of river training works in Burma, it was decided to incorporate the principles of a system used in that country, and to modify it to the special requirements as an anti-malaria control measure.

The low water channels of the Rangoon River and its tributaries are controlled by means of low bamboo stake fences in order to facilitate the rafting of logs from the teak forests. The application of this system on many miles of the Burmese rivers is a definitely established practice and has proved to be an inexpensive and reliable means of preventing shoaling in the channels; it has also proved effective for dispersing the silt brought down by the rivers and in meeting the contingency of silt disposal.

The system as described by LEETE (1924) was advocated by WEIDEMANN in 1877 but was not put into practice until 37 years later, owing chiefly to the lack of co-operation between the various Government Departments concerned, and to the fact that the previous history of the river work had been so discouraging. It was found extremely difficult to convince the higher authorities of the possibilities of doing anything of permanent use, with the result that little was done until 1914. It appears, however, that as the work of clearing the Mingyi Chaung had proved such an unqualified success, the whole attitude towards the river problem changed. It had demonstrated how the Rangoon River could be successfully regulated, and so convinced the Government of Burma that they were prepared to accord sanction to the more ambitious proposals put forward in 1915, and to grant the services of a professional engineer to put the work of river improvement upon a sound basis. Channel control in Burma has become such a routine matter that it attracts but little outside attention.

The method comprises the construction of low parallel bamboo stake fences along both sides of the dry weather channel; these are designed to offer just sufficient obstruction to the flow to cause the suspended silt, carried by the water, to be gradually deposited and in so doing, to produce an increase in the level of the side banks, due to these deposits.

The Burma shallow fences are made of pointed bamboos from 5 to 6 feet in length, driven about 9 inches apart and projecting a matter of 3 feet above ground. They are lashed to horizontal bamboos by coir rope at the intersections, about 6 inches from the top of the stakes. Where the fences cross side channels they are kept somewhat higher and are specially strutted. Where bad bends occur these are eliminated by cuts protected by additional strutting. Pilot channels are occasionally excavated in order to expedite the realignment at deviations. The fences are extended slightly beyond the limit required for silt deposit and the tops of the stakes dressed to conform to a steady slope.

spur dikes alone, by spur dikes of other types, and with the aid of other materials. In order that work of this nature may be carried out upon an extensive scale, it is essential that simplicity of construction be observed and that the cost of such works shall not be prohibitive.

It is necessary that a thorough inspection should be made of the sections to be taken up and the clearing of debris, fallen trees, etc., taken in hand without delay, as until this is done a final survey cannot be made.

The importance of primary clearing cannot be over-estimated, as a considerable amount of the pooling, particularly in the tributaries, is due to this cause alone, and in many instances nothing more is necessary to permit the river carrying out its natural functions.

The draining of pools cannot always be effected economically, but when possible, the water area should be reduced by drainage, preparatory to filling and sealing.

The destruction of islands, where these interfere with natural flow conditions, should where possible be done by the flood waters, the islands being scored by narrow channels to encourage erosion. Back bays formed by the side tracking of flood waters, may in many instances be reclaimed by spur dikes and specially strutted closure fences.

The eradication of malaria by any known means is often a protracted and difficult problem requiring patience and perseverance, but so long as temporary measures constitute the chief method of control in river beds little permanent improvement can be expected in the malaria conditions in the vicinity of rivers.

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A STUDY OF EIGHTY-FIVE CASES OF *STRONGYLOIDES STERCORALIS* INFECTION, WITH SPECIAL REFERENCE TO ABDOMINAL PAIN.

BY

E. HAROLD HINMAN, Ph.D.*

From the Department of Tropical Medicine, Louisiana State University Medical Centre, New Orleans, U.S.A.

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The records of the State Charity Hospital at New Orleans show that during the past 6 years (1930-1936), sixty-six cases of infection with *Strongyloides stercoralis* have occurred.   In all of these the discharge diagnosis has been so listed and a study of their case histories would seem to indicate that the complaints which brought them to the hospital might be directly attributable to this infection. An additional nineteen cases have been studied where the discovery of the parasite was incidental to admssion to the hospital for some other disease, or (in five instances) in clinic patients.   It is due to the courtesy of medical students that most of these latter cases have been called to the attention of the writer.   Undoubtedly, many other cases have occurred during this period, but since no system of cross-indexing exists within the record room, the histories could not be located. The writer has been especially interested in the blood picture in this infection, and has had opportunity to make blood counts on a considerable number of these patients.   In every instance, stool examination revealed the presence of rhabditi-form larvae of this parasite.   Both a clinical and laboratory analysis of these histories is herewith presented.

ETIOLOGICAL FACTORS.

The distribution by sex was fifty-nine males to twenty-six females. This preponderance of males is shown both in the adults and children of the series. In sixteen individuals, 12 years or under, there were ten boys and six girls. The age incidence is shown in Table I.

TABLE I.
AGE INCIDENCE.

0– 5 years	...	2	20–29 years	...	17	50–59 years	...	4
6–12 „	...	14	30–39 „	...	14	60–69 „	...	1
13–19 „	...	24	40–49 „	...	7	Unknown	...	2

Almost 50 per cent. of the cases occurred in individuals under 20 years of age. FAUST (1931) found the highest incidence amongst cases in Panama to be in the 11 to 15 year group and that there was then a slight decline after which it remained approximately constant.

A study of the admission date indicates that the late summer and early autumn show the highest incidence. Forty-four (more than one-half) of the cases were admitted during the 4 months of July-October, inclusive, see Table II.

TABLE II.
ADMISSION DATE.

January	...	...	4	May	...	...	8	September	...	8
February		...	5	June	...	...	0	October	...	10
March	...	...	3	July	...	...	17	November	...	8
April	...	...	8	August	...	...	10	December	...	4

The explanation of this seasonal incidence is difficult. A subsequent table will show that the average duration of symptoms is above 8 months, due mainly to the fact that several patients gave histories of duration of a number of years. It is possible that a number of the patients acquired the infection in the spring or early summer and within a short time began to have manifestations of their infection.

The occupation of the group is shown in Table III.

TABLE III.
OCCUPATION.

School children	...	28	Labourers	...	11	Fishermen	...	4
Farmers	...	15	Skilled labourers	...	7	Miscellaneous or none		5
Housewives	...	15						

It appears that school children, farmers, housewives and common labourers are most susceptible to this infection, due no doubt to more frequent exposure to contaminated soil.

The distribution of cases according to geographical location within the state is shown in Table IV.

TABLE IV.
DISTRIBUTION BY PARISHES.

Allen	...	...	1	Jefferson	...	3	St. Mary	...	1
Ascension	...	4	Jeff. Davis	...	1	St. Tammany	...	2
Assumption	...	4	La Fourche	...	5	Tangipahoa	...	9
Avoyelles	...	6	Livingston	...	3	Terrebonne	...	7
Calcasieu	...	3	Morehouse	...	1	Vermilion	...	2
E. Baton Rouge	...	2	Orleans ...	...	8	Washington	...	5
Iberia	...	...	5	Pointe Coupe	...	1	Unknown	....	7
Iberville ...	...	4	Rapides ...	...	1		

The great majority of the cases occurred in the Mississippi Alluvial Plain or Coastal Plain area. Plotting of the cases by parishes on a map revealed a rather irregular distribution within these two areas. Unfortunately, the series is not large enough to give significant findings on this matter, but apparently soil factors do not limit the distribution of this parasite.

The distribution by colour is shown in Table V.

TABLE V.

White	...	...	69	White males	...	48	Coloured males ...	11
Coloured	...	16	White females	...	21	Coloured females	5

This shows more than four times as many white patients as coloured. SANDGROUND (1926) quotes FÜLLEBORN that clinical symptoms are much more rarely associated with *Strongyloides* infection among negroes and other natives of the tropics than they are among white people. Thus a survey among the general population might show a more equal distribution according to colour than this analysis of hospitalized cases.

THE HISTORY.

In analyzing the histories of these cases, the chief complaint which brought the patient to the hospital is of considerable interest. Table VI lists a summary of these. It should be pointed out that frequently patients mentioned more than one symptom as their chief complaint.

TABLE VI.
CHIEF COMPLAINT.

Abdominal pain (total 44)				Loss of weight	... ...	7
Cramps	... ...	...	12	Vomiting (with or without nausea)	5
Epigastric ...	...	...	13	Diarrhoea	... ...	10
Right side ...	...	...	5	Bloody diarrhoea	... ...	4
Left side ...	...	...	1	Malaise	... ...	4
Right lower quadrant	...	7	Fever	... ...	4
Right upper quadrant	...	1	Constipation	... ...	1
Left upper quadrant	...	1	Indigestion ...	... ...	2
Lower abdomen ...	...	2	Weakness	... ...	4
Upper abdomen ...	...	2	Miscellaneous	... ...	9

It is, therefore, apparent that forty-four, or more than 50 per cent. of these cases suffered abdominal pain which was diffuse, cramping in nature or was chiefly localized in the epigastric region or right lower quadrant. Diarrhoea was the chief complaint in fourteen patients of whom four had bloody evacuations. Malaise, fever, weakness etc., were less frequent presenting complaints. Further considerations of the histories has been summarized in Table VII.

TABLE VII.
THE HISTORY IN EIGHTY-FIVE CASES.

Average duration of symptoms 8·9 months.			
	Cases		Cases
History of diarrhoea	26	Nausea only	2
Bloody diarrhoea	14	Anorexia	13
Mucus in stool	10	Headache	12
Diarrhoea and constipation	7	Malaise	9
Average number of stools per day		Indigestion	8
(diarrhoeic cases) 6·1		Distension and flatulence	9
Constipation only	10	Weakness	12
Tenesmus	5	Loss of weight	26
Abdominal distress	46	Average number of pounds lost	
Localized abdominal tenderness	2	(where stated) 14·5	
Nausea and vomiting	23	Recurrent attacks	8
		Treated previously	2

Table VII reveals the fact that upon further questioning, a total of twenty-six patients gave a history of diarrhoea, of whom fourteen had a bloody diarrhoea some time during the course of their present illness. Seven had an alternating diarrhoea and constipation while ten had constipation only, and ten had mucus in the stool. Abdominal pain or distress was present in forty-six cases or in only two additional cases where it was not mentioned as the chief complaint. Nausea and vomiting and loss of weight were prominent features in this series. Anorexia, malaise, headache, indigestion, distension and flatulence were not infrequent complaints. But it must be remembered that the most striking symptom was abdominal pain and distress.

PHYSICAL EXAMINATION.

Upon physical examination localized abdominal tenderness was present in twenty-nine patients and located as shown in Table VIII. In three patients there was more than one area of tenderness.

TABLE VIII.
LOCALIZED ABDOMINAL TENDERNESS.

Right upper quadrant	3	Left lower quadrant	3	Umbilical	2
Right lower quadrant	8	Right side	1	Lower abdomen	5
Left upper quadrant	1	Epigastric	6	Diffuse	3

It is interesting to compare the location of the tenderness as found by physical examination with the localization of the pain as stated in the patient's history. It is probably true that the areas given in the physical examination are more accurate. In the history more than half of the patients stated that the

distress was either diffuse (cramping) or epigastric and a much smaller group localized it in any of the four abdominal quadrants. Upon physical examination, however, there were found areas of tenderness in one of the four abdominal quadrants in fifteen out of twenty-nine patients.

The highest temperature during hospitalization was above 101° F. in only six patients in the series.

TABLE IX.
HIGHEST TEMPERATURE.

Less than 99·3° F....	43	100·1–101° F.	... 14	Unknown	6
99·3–100°	16	Above 101°	... 6		

LABORATORY STUDIES.

Stool examination revealed *Strongyloides* larvae in every case. In forty patients other intestinal parasites were present, the most common being *Trichocephalus trichiurus* which was found in seventeen instances.

Gastric analysis was performed in thirteen cases. In three of these hypochlorhydria was found, with two showing an achlorhydria. (Table X.)

LEVIN (1930) lists the results of gastric analysis in ten cases, of which two showed achlorhydria.

TABLE X.
GASTRIC ANALYSIS.

Case No.	Free HCl.	Total Acid.	Case No.	Free HCl.	Total Acid.
35	5	15	61	25	55
43	0	12	66	45	66
48	60	80	67	50	70
50	45	78	68	55	75
51	0	4	72	32	50
53	38	55	73	55	75
54	25	62			

In forty-seven cases differential blood studies were carried out (Table XI).

TABLE XI.
BLOOD STUDIES.

Total red blood corpuscles (per c.mm.).		Haemoglobin.		Total white blood corpuscles (per c.mm.).	
Less than 2 million ...	1	40–49 per cent.	5	Less than 5,000 ...	1
2–2·9 million	3	50–59 „ ...	2	5,000–7,500	7
3–3·9 „	8	60–69 „ ...	6	7,500–10,000	25
4–4·9 „	13	70–79 „ ...	8	10,000–15,000	4
5+ „	5	80–90 „ ...	4	Over 15,000	2
		90+ „ ...	3		
Total cases	30		28		39

Differential Count in 47 Cases.

Polymorphonuclears.		Lymphocytes.		Eosinophils.	
Less than 40 per cent.	4	Less than 20 per cent.	11	0–2 per cent. ...	11
40–50 per cent. ...	2	20–30 per cent. ...	14	3–6 „ ...	12
51–60 „ ...	19	31–40 „ ...	17	7–10 „ ...	10
61–70 „ ...	9	41–50 „ ...	4	11–15 „ ...	7
71–80 „ ...	12	50+ „ ...	1	16–20 „ ...	4
81+ „ ...	1			21–25 „ ...	3
	47		47		47

Average eosinophils 8·6 per cent.

The majority of these cases showed a moderate secondary anaemia. The total leucocyte count was rarely increased—only six having a count above 10,000 per c.mm. While there was a slight increase in the polymorphonuclears, thirteen being above 70 per cent., the most striking characteristic of the differential count was the presence of an average eosinophilia of 8·6 per cent. in forty-seven cases. Twenty-four cases had 7 per cent. or over of eosinophils. The highest count was 24 per cent. In LEVIN's (1930) series the average was 2 to 8 per cent. with a single case of 13 per cent. HENSEN (1923) found as high as 82·6 per cent. eosinophilia in a human case. According to SANDGROUND (1926) BRAU found an eosinophilia ranging from 25 to 69 per cent. SANDGROUND (1926) observed only slight eosinophilia in four dogs experimentally infected with this organism.

DIAGNOSIS.

The diagnosis of *Strongyloides stercoralis* becomes exceedingly difficult where stool examination is not performed routinely. Five of these cases were admitted with a tentative diagnosis of appendicitis and the interns considered such a diagnosis in a number of other cases. Cholecystitis was suspected in a few other instances.

The varied distribution of abdominal pain and even tenderness upon palpation in these patients stresses the importance of faecal examination whenever these symptoms are manifest.

While this series presented an average eosinophilia of only 8·6 per cent., yet more than a fourth of them showed a differential count of over 10 per cent. Animal parasites have long been considered an important cause of eosinophilia, yet in the writer's experience this is often small or inconstant in many of the helminthic infections, except during that period when migration is occurring through the lungs. Eosinophilia appears to be more constant in *Trichinella* and *Strongyloides* infections than in other intestinal helminths.

TREATMENT.

Records of these cases show that forty-six patients were treated with at least one course of gentian violet. Unfortunately, many of these patients were discharged from the hospital without the record sheet stating whether or not a follow-up stool examination had been performed. It is, therefore, unwise to draw any conclusions as to the efficacy of this drug. None of the patients was readmitted to the hospital subsequent to this course of therapy, and there was no mortality in the series. It is, therefore, presumed that their symptoms were relieved as a result of this treatment. FAUST (1930) was the first to use this drug in the United States. A great variety of anthelmintics were tried in the other patients of this group.

DISCUSSION.

The pathogenicity of *Strongyloides stercoralis* has been questioned by certain writers but more recent work would seem convincing that this parasite is truly pathogenic, see FAUST (1932) for historical discussion. The life cycle of the parasite is such as to suggest pathology within the host. The adult female worms live primarily in the mucosa of the upper levels of the small intestine and produce their eggs in this location. The latter work their way out into the lumen and before being passed in the faeces hatch into the rhabditiform larvae. After development in the soil, the infective filariform stage is reached and when these come in contact with the skin are able to penetrate the host and eventually reach the pulmonary capillaries where they penetrate into the alveoli and bronchioles. Subsequently, they migrate to the epiglottis and then reach the small intestine where development is completed.

Therefore, one might expect tissue reactions at the site of invasion (the skin), within the lungs, and within the duodenum, jejunum and ileum. The work of FAUST (1935) indicates that such is the case.

The symptom which was first associated with parasitism by *Strongyloides* was uncontrollable diarrhoea. While diarrhoea was present in twenty-six cases of this series, it was the presenting complaint in only ten instances. SANDGROUND (1926) believes that the diarrhoea in *Strongyloides* infection results either from mechanical irritation due to the burrowing activities of the parasite in the wall of the small intestine or from toxins elaborated by the parasite. LEVIN (1930) states that there are three clinical types of cases : (1) heavy infestation with *Strongyloides* and a chronic diarrhoea—a rare type ; (2) medium or mild infection with periodic mild diarrhoea of short duration—a more frequent type ; (3) a mild, medium or heavy infection with constipation—the most common type.

Two patients stated that, preceding the onset of diarrhoea, they had been troubled by cough. It is possible that this represented pulmonary irritation due to presence of the migrating larvae.

Abdominal pain and distress was the symptom for which forty-four of these patients sought relief. The extreme variability of location and character of this pain makes it essential to perform stool examination in all cases where these symptoms present. By this simple procedure parasitic infections can at once be excluded from difficult differential diagnosis. The writer does not wish to over-emphasize the question of abdominal pain or tenderness, but from careful reading of the histories, one cannot help but be impressed by its importance.

SUMMARY.

1. An analysis of eighty-five cases of *Strongyloides stercoralis* infection is presented. Diagnosis was proved in each case by stool examination.

2. Among etiological factors studied it was found that there was a marked predominance in the male sex, in the white race, and in the second, third and fourth decades of life. Seasonal distribution and occupation appear to be of some significance.

3. The most frequent presenting complaint was abdominal pain. Diarrhoea was less common.

4. The history revealed that in addition to abdominal distress and diarrhoea, nausea and vomiting, headache, anorexia, loss of weight, weakness, malaise, constipation, distension and flatulence were frequent symptoms.

5. Physical examination revealed localized abdominal tenderness in $34 \cdot 1$ per cent. of the cases. Fever above 101° F. was infrequent.

6. Laboratory studies showed three of the thirteen cases with hypochlorhydria, a moderate secondary anemia, rarely leucocytosis, and an average of $8 \cdot 6$ per cent. eosinophils in forty-seven cases.

7. The diagnosis of *Strongyloides* infection must be considered in all cases presenting abdominal distress, vague dyspepsia, diarrhoea or eosinophilia. Stool examination in most cases will readily exclude this condition.

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FIÈVRE BOUTONNEUSE IN SPAIN AND ITS EXPERIMENTAL TRANSMISSION BY TICKS.

A PRELIMINARY NOTE

BY

CALONGE RUIZ, M.D., AND ORTIZ DE LANDAZURI, M.D.,

Madrid.

In the Hospital for Infectious Diseases in Madrid, of which Dr. M. Tapia is Director, there have been during the last few years eighty cases of fièvre boutonneuse or eruptive fever, transmitted by the dog-tick *Rhipicephalus*
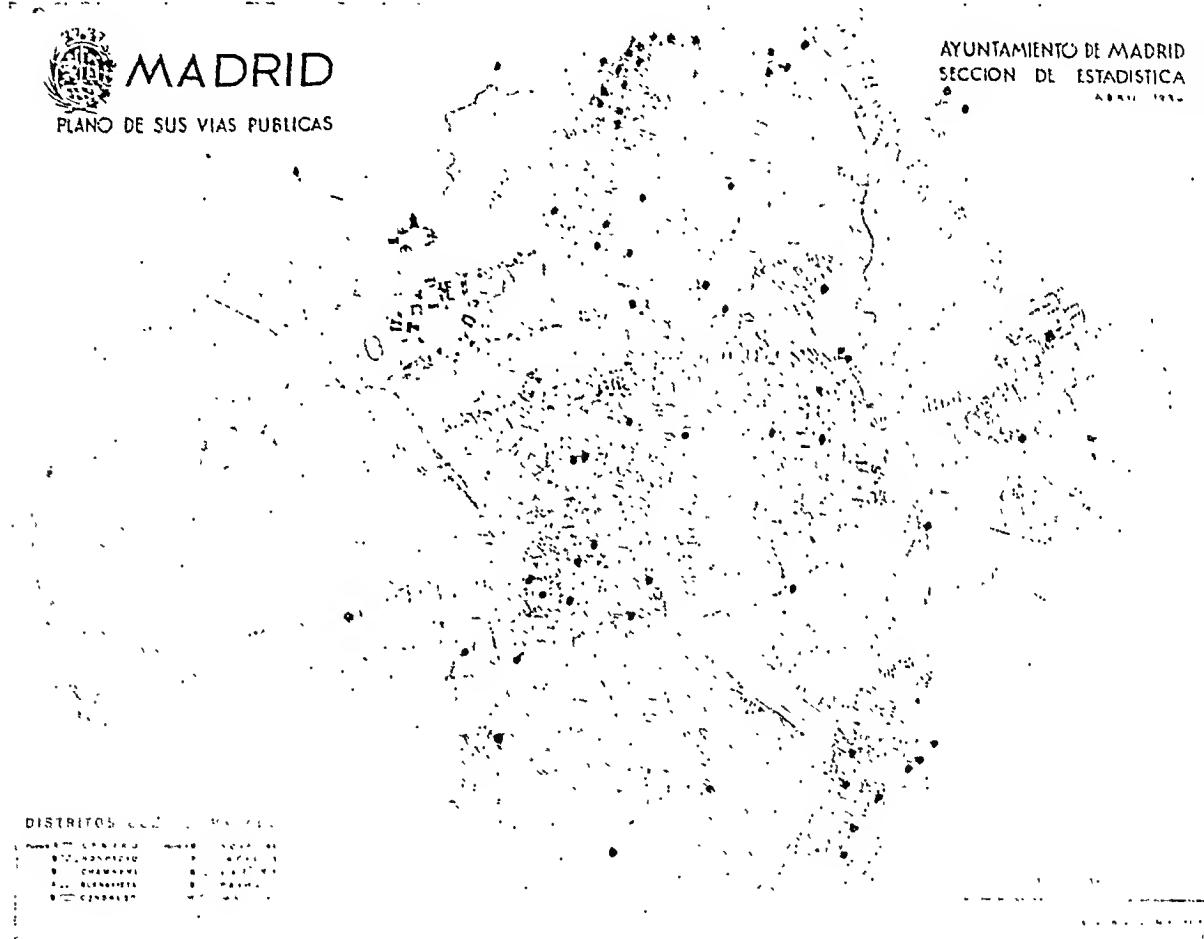
DISTRIBUTION OF CASES OF FIÈVRE BOUTONNEUSE IN MADRID.

sanguineus. Cases have also been observed in other parts of Spain. On the suggestion of Dr. Tapia, who investigated this disease, the Permanent Commission for Sanitary Research of the Ministry of Labour and Public Health, which until November last was presided over by Professor Pittaluga, made itself

responsible for the expenses and supervision of the research work that we have carried out in the Hospital for Infectious Diseases and in the National Institute of Health, which is still under the direction of Dr. TAPIA.

First of all we studied the distribution of cases in the precincts of the city of Madrid and its outskirts (see Map), following them up from their focus of origin. As regards the distribution of this disease there are two principal foci, the first at Tetuan de las Victorias, the second at Puente de Vallecas (which are the two poorest working-class suburbs). Other cases are distributed irregularly throughout the city.

Dog-ticks were collected in the houses where the cases occurred and extracts of crushed ticks were inoculated into young guineapigs (nine were inoculated). The guineapigs were kept under daily observation : seven out of the nine cases had raised temperature. The postmortem and particularly the pathological examination of the testicles did not reveal any appreciable lesions.

Extracts of the testicles and of the brain of three guineapigs of the first lot were inoculated into a second lot of guineapigs which are at present being examined. We also inoculated the blood of a patient suffering from fièvre boutonneuse when the eruption was at its height as well as the contents of some pustules from the site of inoculation in guineapigs and mice.

Dog-ticks bred in the laboratory produced eggs and larvae in which one could recognize, in small quantities, micro-organisms of the rickettsia type.

The blood of two patients was inoculated (on the eighth day of the illness) into two inmates of Professor LAFORA's Hospital for Mental Diseases. A typical eruption appeared 20 days after inoculation. A third person who had been inoculated showed no symptoms of the disease. The blood of these patients was also inoculated into some other individuals (see Figs. 1 and 2). At the same time, and we believe for the first time (circumstances at the moment in Spain unfortunately prevent us from making any bibliographical investigations) we obtained, by inoculating an extract of ticks, an eruption typical of fièvre boutonneuse in a female patient in the same Hospital for Mental Diseases. This manifestation, which we believe corresponds to the clinical symptom of fièvre boutonneuse produced experimentally, occurred 6 days after inoculation (see Fig. 3). The blood of this patient was inoculated into another person under the same conditions. In view of the present state of affairs in Madrid we do not know the result of this inoculation.

This article must only be regarded as a preliminary summary of the results of these investigations. We apologize for this incomplete account which we are now submitting (20th October, 1936) as we believe we shall not be able to pursue our work and to publish it *in extenso* owing to the tragedy which is disturbing normal life in Spain.

[ED.—Similar experiments have been carried out in Roumania by COMBIESCO and ZOTTA who have infected volunteers by the inoculation of blood from human cases, of emulsions of ticks removed from dogs, and emulsions of larvae hatched from eggs laid by ticks harbouring the virus.—*Trop. Dis. Bull.* (1933), xxx.]

FIG. 1.

FIG. 2.

FIG. 3.

ERUPTION IN EXPERIMENTAL FIÈVRE BOUTONNEUSE.

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A DENGUE-LIKE FEVER IN THE GOLD COAST.

BY

F. M. PURCELL, M.D., M.R.C.P.I., D.T.M. & H.,

Medical Officer, Oda, Gold Coast.

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Although dengue fever has been diagnosed in the Gold Coast, the only clinical account which has been published is that of a case described by the writer.*   The distribution of the disease is not known and no insect vector has been incriminated.   Having had an opportunity of studying a number of cases of dengue it seems that a brief description of these will be of use to other observers.

There prevails in Akim-Oda, in the south of the colony and elsewhere in the forest country a form of dengue fever which affects Europeans, in many cases soon after their arrival in the country.   Recent experience proves that native Africans also may become infected.

In the case reported in 1932, the patient suffered from fever which declined in the morning of the 4th day when a profuse rash appeared on the chest and abdomen.   By the 5th day the rash had completely disappeared.   There was a rise in temperature on the 6th day after which the patient was normal.   Though a number of other cases have been seen it is unfortunate that, except

* PURCELL, F. M.   (1932).   Notes of a case of fever in some respects resembling mild dengue fever.   *West Afr. Med. J.*, vi, 6.

for this one, of which the full details are given in the original paper, the observations have been scanty owing to the fact that the patients have been seen late in the disease and have not been treated in hospital. However, sufficient data have been obtained to warrant a description of the characteristics of the disease.

EPIDEMIOLOGY.

Incidence.—It has been observed that any European stationed in the forest country is liable to acquire infection. Immigrant Africans also are susceptible.

Distribution.—The disease is prevalent widely in the forest country, cases having been noted in areas as far apart as Sunyani in the west, Ho in the east and Assuansi in the south. It is noteworthy that the disease does not occur in the coastal towns nor in widely cleared areas such as Kumasi. It seems to be confined to the forest regions in the rainy belt. Native villages, such as Assuansi and Jeydem, are endemic foci. This must apply also to other villages; for the disease appears to be endemic throughout Akim, as officers acquire infection when on trek. In some years the disease takes on epidemic characters, as in 1929, when cases occurred around Oda, at Akrokerri in Ashanti, and in the Kpandu-Ho country in the east. Comparison with yellow fever suggests a focus of infection in the native Africans.

Season.—There are two rainy seasons in the forest country (*a*) late March to July and (*b*) September to November. The other months are dry, but a wholly rainless month is exceptional. The season of highest incidence appears to be that of the first rains. Cases occur, however, in September but not in the other months.

Vector.—No definite information concerning the actual vector can be given as no investigations have been carried out. A midge or sand-fly (*Culicoides grahami*) is very prevalent in the endemic areas; it is an avid feeder. The mosquito *Aëdes aegypti* is widely distributed. It is the transmitter of yellow fever in the Gold Coast and occurs also in the dengue areas, yet the absence of dengue from the coastal towns is remarkable, although *Aëdes aegypti* occurs there.

CLINICAL DESCRIPTION.

The occurrence of the disease in individuals a day or two after return to Accra after a brief visit to Assuansi suggests a short incubation period of a few days.

The onset of the disease is mild, or severe, with fever and general malaise. The temperature is highest at the onset and is remittent until the rash appears on the 4th day. It may rise slightly on the following day. The highest temperature recorded was 103° F.

The pulse, usually slow in proportion to the temperature, was remarkably slow in one case. The illness lasts from 3 to 10 days (including the disappearance of the rash), according to the severity of the attack.

Apart from the feeling of general malaise and prostration, the symptoms complained of were pains behind the eyeballs and slight photophobia, pains in the muscles and pains in the joints, particularly the finger joints. Itching, affecting the palms and soles of the feet, was present in a few cases but absent in most.

The rash appears usually on the 4th day and is fully developed in 24 hours. In many cases, however, the rash appears earlier and may in fact be the first sign observed. The eruption resembles that of scarlet fever ; it consists of pin-head sized red points surrounded by an ill-defined pink area. These points or papules can be felt by the hand. In very mild cases the papules are absent or indefinite. On the feet the rash may have a petechial character but this may be influenced by activity in ambulatory cases. The skin of the face may be blotchy pink and rough. In the African the skin points may take the form of soft, tiny papules or vesico-papules. On a pale and delicate skin the rash is seen to affect all areas, including the face, the palms of the hands and soles of the feet. It is distributed in order of profusion, on the abdomen, chest, back, arms, thighs ; it is scantiest on the forearms and legs. On the exposed, pigmented thickened skin of the out-of-door European it may not be visible on the face, forearms or legs. Nearly always the rash is profuse and it is constantly most marked on the sides of the abdomen and, therefore, easily identified. In mild cases, or in a second infection, a pink macular rash may appear and vanish in less than 24 hours. Typically the rash develops fully by the 2nd day and fades slowly to disappear as a rule in about 5 days.

The blood changes were not studied in detail but in one case a fall in the number of polynuclear cells was noted. Lymphatic gland enlargement was reported in one severe case. Albuminuria and catarrhal symptoms were not noted while gastro-intestinal upset was rarely seen.

In general it may be concluded that the disease occurs in four degrees of severity. There are (1) very mild cases in which the patient is hardly aware of being unwell till the rash appears ; (2) mild cases showing slight but definite malaise for 3 or 4 days before the appearance of the typical profuse rash ; (3) moderately severe cases with a temperature reaching 102 to 103° F. associated with pains in the muscles and joints ; and (4) severe cases with high temperature, severe pains, prostration, adenitis and alimentary upset.

Prognosis is always favourable and nothing is known of any complications or sequelae. There is no treatment beyond that to relieve the symptoms. Salicylates proved ineffective to control the pains.

DIAGNOSIS.

Moderate fever of 4 days' duration followed by a profuse pink rash on the trunk on the 4th day is characteristic of dengue fever in the endemic area. The disease differs from classical dengue in that the rash occurs early and not

after a period of remission.   A rash affecting the skin near the scalp and appearing on the first day of fever and associated with enlargement of glands in the vicinity should warrant a diagnosis of German measles to which, however, adult Europeans are probably usually immune, though at least one case of German measles has been diagnosed by my colleague.   Absence of catarrhal symptoms, conjunctivitis, bronchitis or sore throat should exclude measles and scarlatina. In practice in the forest country the disease in a European has to be distinguished from malaria and from mild yellow fever ; in an adult African from malaria, yellow fever, chicken pox, variola ; and in the African child from measles and " sweating fever " (miliary fever).   A European suffering from mild dengue assumes he is suffering from malaria as the subjective symptoms are similar.   Blood examinations and failure of response to quinine exclude malaria. Post-orbital or other pains or prostration out of proportion to the temperature raise suspicion of dengue.   A severe case of dengue might easily be mistaken for yellow fever in which however definite evidence of nephritis with increasing albuminuria soon become evident.   As a final diagnosis is often impossible during the first three important days of the illness precautionary measures should be adopted by keeping the mosquito net down from the onset.

Because of their transmission by the same mosquito investigators have considered the relationship of dengue and yellow fever.   Observations indicate that the two diseases are distinct, a conclusion which is borne out by the experience of the writer who suffered from yellow fever in 1931 and from dengue fever in 1936.   It seems probable that dengue fever must be endemic in the native community in the rainy country.   The widespread and seasonal infection of Europeans suggests this.   The suspicion is made stronger by the analogy of yellow fever.   On that account it was of interest to encounter three cases in African adults especially as in each case the sufferer was an immigrant to the forest country.   The diagnosis of the disease in childhood is beset with many difficulties, so much so that up to the present there has been failure to diagnose dengue in a child.

OBITUARY.

SIR HENRY WELLCOME,
LL.D. (EDIN.), HON. F.R.C.S. (ENG.), F.R.S., F.S.A.
1853—1936.

HENRY WELLCOME, Honorary Fellow of the Royal Society of Tropical Medicine and Hygiene, died in London on 25th July, 1936, at the age of 82. Famous throughout the world as chief of the great firm of Burroughs Wellcome and Co., of which he became the sole proprietor, he was equally, if not better known for his life-long devotion to medical science, to the furtherance of which in one or other of its branches, particularly tropical medicine, he dedicated his life and practically all his wealth.

He was born in 1853 in a log cabin near Almond, Wisconsin, where his father, an itinerant missionary, was working amongst the Dakota Indian tribes. At the age of 15 he commenced work as a pharmacist in Rochester, but 3 years later moved to Philadelphia where, under the influence of Dr. WILLIAM WORRALL MAYO, father of the famous MAYO Brothers, he studied at the College of Pharmacy, from which he graduated in 1874.

In his capacity of a pharmacist, he travelled the length and breadth of the United States and eventually found his way to South America, where he visited the cinchona forests and formed his own opinions on the subject of quinine production, a problem in which he always remained profoundly interested. It was there that he had his first experiences of tropical medicine which was to become one of the chief interests of his life.

In 1880, he came to England and founded, with Mr. S. M. BURROUGHS, the firm which soon became famous for the quality of its pharmaceutical products manufactured at the great works at Dartford, Kent. The very nature of this business kept him in touch with medical developments and with a far-reaching insight he adopted anything of definite therapeutic promise and offered it to the medical profession for the treatment of the sick. He soon began to realise that mere production was not enough to ensure complete success and that for real progress this had to be supported and advanced by scientific research. Some provision for this had already been made at the works at Dartford ; but in order that research could be carried out without the hindrances occasioned by immediate productive demands he founded the Chemical Research Labora-

tories in 1896.   These have been largely occupied in the study of the chemistry of medicinal substances many of them of use in the treatment of tropical diseases ; and also in the wider problem of the relationship of chemical constitution to therapeutic or physiological action.

Two years earlier, in 1894, appreciating the possibilities of serum therapy, then in its infancy, HENRY WELLCOME had founded the Physiological Research Laboratories, now at Beckenham, Kent, for the purpose of preparing sera and biological products, and for investigations in the fields of immunology and pharmacology.   These laboratories are now known everywhere not only for the high standard of the sera and similar therapeutic agents prepared by them but above all for the research work carried out there and for the many original contributions made to knowledge of the fundamental problems of immunology and kindred subjects.   Though these laboratories have an indirect connection with tropical medicine WELLCOME's leaning towards this branch of medicine took definite shape after his visits to the Sudan and his realization of the medical needs of that country. He equipped the research laboratories in the Gordon College, Khartoum, and these became known as the Wellcome Tropical Research Laboratories.   Under the able directorship of the late Sir ANDREW BALFOUR, they carried out investigations of the greatest benefit to the Sudan, and quickly established an international reputation.   The beautifully illustrated reports, the issue of which was made possible by the personal interest of the founder of the laboratories, are known and consulted in every part of the world. These laboratories, though now replaced by another organization, were largely responsible for the general recognition of the value of research to the medical and sanitary development of tropical Africa and other countries.   When the foundation of the Gorgas Memorial Laboratories at Panama was contemplated, WELLCOME's experience based on the achievements of the Khartoum Laboratories was of the greatest value in deciding the committee entrusted with the examination of the project to recommend in favour of the scheme.

In what may appear to be a completely different direction, Africa held a peculiar fascination for HENRY WELLCOME who found there an opportunity of gratifying a lifelong interest in archaeology and primitive medicine.   His discovery of certain remains in the Sennar Province of the Sudan led him to undertake the systematic investigation of the sites at Jebel Moya.   This, continued through many years often under his direct supervision, has yielded antiquities the actual value and significance of which have not yet been assessed. His intense interest in archaeology and ethnology led him to collect a vast amount of material bearing on the medical development of mankind.   A part of this collection, much of it from the tropics, formed the basis of the Historical Medical Museum, opened in a building in Wigmore Street, in 1913, at the time of the International Medical Congress.   Here, as many will remember, WELLCOME was never happier than when entertaining amongst his unique exhibits those

Henry Wolcaws

who had shared his archaeological and historical tastes. At the moment the entire collection is being reviewed and will eventually form a more complete exhibit in the Wellcome Research Institution. It had been his wish to supervise personally the final arrangement of this museum, but failing health denied him the fulfilment of his life's ambition. A similar interest and one of more recent date concerned the Archaeological Research Expedition organized in 1932 to examine Tel Duweir in Palestine, which has already identified the site as that of the Biblical Lachish and has produced the now famous Lachish letters of the time of Jeremiah.

Returning now to matters more directly concerned with tropical medicine, mention must be made of the foundation in 1913 of the Bureau of Scientific Research under the direction of the late Sir ANDREW BALFOUR, who retired from his post as head of the Khartoum Laboratories. The Bureau was designed to co-ordinate the various laboratories and museums HENRY WELLCOME had founded, to carry out research in subjects of tropical medical interest and generally to play a part in furthering knowledge of tropical medicine and hygiene throughout the world. Housed at first in adapted buildings in Endsleigh Gardens, the Bureau of Scientific Research and the Museum of Medical Science, which was established in connection with it, quickly outgrew available space, and in 1931, he made the final decision to pull down the old premises and, on a more extended site, to build the Wellcome Research Institution which now stands in Euston Road as one of the most prominent and distinctive architectural features of this part of London. Here have been gathered the Bureau of Scientific Research, the Chemical Research Laboratories and the Museum of Medical Science while, as already intimated, the Historical Medical Museum now in process of arrangement should before long be open to visitors. The foundation stone of this building was laid 25th November, 1931, by the late Lord MOYNIHAN, who referred to it " as a corner-stone of the life-work of the man who has done as much as any man in this or any other country to advance both the Science and Art of Medicine."

As would be expected, HENRY WELLCOME, with his world-wide interests and connections was acquainted with most of the prominent medical men of his time, many of whom were on terms of intimate friendship with him. In the field of tropical medicine he perhaps valued the friendship of MANSON and GORGAS before all others and he often spoke of these pioneers in terms of the greatest admiration. He enjoyed a long friendship with STANLEY which, no doubt, accounted for his intense interest in the welfare and development of the Dark Continent and his whole-hearted support of the Royal African Society of which he became Vice-President. In 1935 the Royal African Society awarded him its Gold Medal—a medal which he himself had founded in 1923, to be given to those who had rendered distinguished service to Africa.

When the Royal Society of Tropical Medicine and Hygiene embarked

upon the scheme for securing a house of its own as a memorial to MANSON, HENRY WELLCOME made a generous donation to the funds of the Society.

In this brief sketch, it has been possible to mention but a few of the many undertakings of this most strenuous life which combined in a remarkable manner both commercial and scientific interests. Having built up his important business WELLCOME employed its profits almost entirely for assisting medical research and other scientific projects. Commerce and science were inevitably linked together in his mind, for progress of the one meant more funds at his disposal for the other. He himself was one of the most abstemious of men. He led a relatively simple, even a spartan, existence and spent upon himself little of the great income to which he was entitled.

Towards the end of his life it became his chief concern to arrange for the continuance of his life's work which was to co-ordinate a great business with scientific research in such a way that the whole should become a self-supporting organization. Accordingly, the first step was taken in 1924, when the Wellcome Foundation was formed, with himself as Governing Director, to unite the business of Burroughs Wellcome and Co. and its various ramifications with the research laboratories and museums he had founded. This organization was in full working order when he died and his Will has ensured that the Wellcome Foundation shall be continued and developed as a self-supporting concern, as was the case during his life ; while any profits which shall remain after the working of the Foundation are, apart from certain minor bequests, to be devoted to other medical and scientific research charities. The whole of the business and scientific interests throughout the world are vested in five trustees while the actual working of the Foundation is the direct concern of a Board of Directors with a Governing Director as the head.

Sir HENRY WELLCOME was a member of numerous societies and was the recipient of many honours and awards. He was knighted by the late KING GEORGE V in 1932 and in the same year was elected a Fellow of the Royal Society in recognition of his many services to science.

C. M. W.

PAPERS.

SIGMOIDOSCOPY IN TROPICAL PRACTICE.
BY
PHILIP MANSON-BAHR, M.D., F.R.C.P.
Physician to the Hospital for Tropical Diseases, Gordon Street, London.

Once before I have had the honour of contributing a paper on sigmoidoscopy to this Society, some 16 years ago, when the appearances of chronic bacillary and amoebic dysentery and their differential diagnosis were described by Dr. A. L. Gregg and myself.[*]

Before proceeding to the practical uses of sigmoidoscopy in diagnosis and treatment of tropical disease, it is necessary to refer to certain principles which

[*] Manson-Bahr, P. H. & Gregg, A. L. (1921). *Trans. R. Soc. trop. Med. & Hyg.*, xiv, 5, 88-91.

should be mastered, if the most is to be gained from the routine use of this valuable procedure. Sigmoidoscopy is a simple and not necessarily a very disagreeable method of examination. It is, therefore, a matter of considerable surprise to find that a method of direct inspection of the mucous membrane and contour of the intestinal tract, which is thus laid bare to the investigator, is not more generally adopted, and the characteristic lesions of the various forms of dysentery more generally understood in tropical practice. The methods of bacteriological and chemical analysis have always appeared to me to be of secondary importance to the study of what may be termed " vital pathology." One cannot imagine a throat specialist making a diagnosis or giving an opinion without employing all the methods of inspection of the throat and air passages at his command by means of the laryngoscope or bronchoscope, and why should not the tropical consultant employ a method even more simple, and perhaps even less disagreeable, for inspecting the anal canal and rectum in cases of bowel disease ?

For local examination of the anus and rectum, the left lateral position is most comfortable and is less embarrassing when it is necessary to make an examination in the consulting room ; but should the patient be very stout, or particularly muscular, the adoption of the more uncomfortable knee-elbow position usually makes the examination easier.

Before inserting any instrument, it is necessary to examine the external parts, the condition of the skin should be noted, and search made for orifices of fistulae. In certain chronic diarrhoeas, such as chronic bacillary dysentery or sprue, the skin at the anal orifice has a peculiar glabrous or leucoplakic appearance, which is quite characteristic. A preliminary digital examination of the anus and anal canal should always be undertaken before proceeding to sigmoidoscopy. Experience has shown that, if this simple procedure is omitted, thrombosed internal piles or small carcinomatous ulcers may easily be missed or overlooked when the sigmoidoscopic tube is being rapidly extruded by sphincteric pressure. The examining finger, covered by a thin finger-stall, causes the minimum amount of pain and discomfort when carefully introduced. The point of the finger should be pressed towards the lateral wall of the anus and slowly inserted, so that lesions within the last $\frac{3}{4}$ inch of the bowel and any excrescences or indurations may be felt.

It is necessary to state that, unless thrombosed, internal haemorrhoids cannot usually be felt nor can their extent be estimated without local inspection.

The indications for sigmoidoscopy may be summarized as follows :—

(1) Complaint of rectal pain, tenesmus, or chronic diarrhoea, muco-purulent or blood-stained stools.

(2) Haemorrhage from the anus, not attributable to piles.

(3) Constipation, alternating with false diarrhoea.

(4) Chronic diarrhoea which does not yield to simple medical treatment.

(5) Blockage of the bowel due to some obscure condition.

It is generally recognized that in practised hands accidents are very rare nowadays through sigmoidoscopic examination ; and, indeed, BENSAUDE,* the author of the well-known monograph on sigmoidoscopy, records that none have ever happened in his experience.

The form of sigmoidoscope most commonly in use in English practice is Lockhart-Mummery's modification of the original Strauss sigmoidoscope. This instrument is 30 cm. long and consists of a tube which is closed posteriorly by a detachable glass window. An essential part of the instrument is a short tube for the attachment of bellows for inflation of the bowel. The instrument is introduced by means of an obturator, which is subsequently withdrawn.

A smaller modification of the sigmoidoscope—the proctoscope—some 6 inches in length may be used for inspection of the rectal canal. Other instruments necessary are a long crocodile forceps for introducing pledglets of cotton wool for wiping the mucous membrane (Patterson's bronchoscopic forceps), a long probe for attachment of swabs for bacteriological purposes, a long-handled spoon on the principles of the Volkmann type, which has been devised for obtaining scrapings from dysenteric and other ulcers for microscopic examination, forceps for obtaining pieces of tissue (biopsy) for microscopic section, an insufflator for blowing in medicated substances, and a snare for the removal of polypi etc.

It is necessary that the tube and obturator should be placed in boiling water for sterilization purposes preparatory to use, but other parts of the sigmoidoscope should not be subjected to heat. The eye pieces require special attention and should always be cleaned with spirit and warmed before use ; otherwise they become fogged and useless.

It is absolutely essential for the study of the finer lesions, such as are seen in amoebic dysentery, that a special magnifying eye-piece, such as Wolfe's, should be employed. Such eye-pieces magnify 2 to 4 diameters, and should be warmed to prevent fogging.

TECHNIQUE OF SIGMOIDOSCOPY.

The rectum is not a straight tube, in spite of its name. When the patient is placed in the genupectoral position, the rectum presents its perineal portion, 2 to 3 cm. in length, directed horizontally forward, and then a second pelvic portion, 9 cm. in length, which is directed antero-posteriorly. The shape of the of the rectal ampulla itself is very variable.

The interior of the rectum is not entirely smooth, because immediately above the sphincter is found a series of longitudinal folds which are known as the columns of Morgagni. In the sphincteric region there are numerous small depressions which represent the openings of the anal mucoid racemose glands. The junction of the sigmoid flexure with the rectum is situated 11 to 13 cm.

*BENSAUDE, R. (1926). *Traité d'Endoscopie, Rectoscopie, Sigmoïdoscopie.* 2nd Ed. Paris : Masson et Cie.

from the anus. The form of the loop at this situation may be compared schematically to the two arms of a V reversed, so that it is never possible to see beyond 32 to 35 cm. from the anus.

The appearance of the mucous membrane and of the bowel in a normal state.

In the normal state the mucous membrane has a uniform rose or rose-red appearance. If anything, it is paler and more yellowish in the region of the sphincter than in the ampulla. However, considerable care must be taken not to attach too much importance to minor changes in the colour of the lower part of the rectum, as aperients or enemata can influence the state of the mucosa in this region to a considerable extent.

Preparation of the patient for sigmoidoscopy.

For the examination to be satisfactory it is most essential that the lower bowel should be as entirely free from faeces as is possible. It is seldom possible to examine a patient without some form of preparation, unless the bowels have been naturally well emptied just previously. In cases with constipation strong cathartics must be used with caution because the cathartic action may be continued and the bowel flooded with liquid faeces.

Not later than 2 p.m. on the day before the examination the patient should be given $\frac{1}{2}$ to $\frac{3}{4}$ oz. of castor oil, and lavage of the bowel with hot water (1 pint or more) should be performed on the morning of the examination. Should the patient be suffering from chronic diarrhoea, then the preliminary aperient should be omitted and no solid food should be eaten on the previous evening.

To deaden pain and discomfort, 5 to 15 min. of tincture of opium should be given $\frac{1}{2}$ to 1 hour before the examination ; but if the diarrhoea is very severe, the injection of $\frac{1}{4}$ grain morphia may be necessary. Luminal, grain 1 to 2, is also useful. Seldom, if ever, unless the condition is very painful, as in ulcerative colitis, does it become necessary to administer an anaesthetic, such as sodium evipan.

This is the practice adopted in the Hospital for Tropical Diseases, but it must be remarked that some authorities prefer to omit any special preparation, holding that the mucous membranes are then seen in their most natural state.

The position in which the patient is placed for sigmoidoscopy is important. Undoubtedly the genupectoral position is the one most favourable from the operator's point of view, but in elderly and obese patients, it is disagreeable and undignified.

The dorsal, or lithotomy, position, with knees raised, is the one most usually employed. It is an advantage when, as sometimes happens, considerable inflation and manipulation of the bowel is required in order to pass the instrument round the various bends encountered.

Before introducing the sigmoidoscope, the tube should be warmed in hot water, but it should be tested on the skin of the hand first, as it must be

remembered that the anus is extremely sensitive to heat. The tube and the anal margins should be lubricated with a glycerine antiseptic jelly, known as KY jelly (Johnson and Johnson).

The ease with which the tube glides over the mucous membrane and enters the pelvic portion of the sigmoid depends upon the skill of the manipulator and especially the application of systematic and gentle insufflation. The best view of minor changes in the mucous membrane is obtained during the process of retraction of the instrument. It is essential that the diagnostician should familiarize himself with the normal appearances of the mucous membrane and of the structures he encounters.

The region of the ampulla of the rectum is provided with valves. At a distance of 7 cm. from the anus two valves cross each other at right angles ; the first of these is known as the valve of Houston ; the second as that of Nélaton (or the coccygeal valve). In 28 per cent. of people a third valve, a superior sacral valve, exists. At 11 to 14 cm. the " recto-sigmoid sphincter," or the " sphincter of O'Beirne " is encountered, and it gives the appearance of the " os uteri " in a normal subject, but it is not seen in the cadaver.

After passing the sphincter the entrance into the sigmoid loop is marked by transverse folds, and at 15 to 20 cm. from the anus, a region is reached where pulsations are conducted from the internal iliac artery, which gives a most characteristic appearance. From 32 to 35 cm. the limits of the ascending loop of the sigmoid are reached, and the well-marked folds of mucous membrane are seen.

LESIONS OF DIAGNOSTIC IMPORTANCE.

The material upon which these statistics have been based has been derived entirely from patients under my care in the Hospital for Tropical Diseases, during the last 17 years. The total of 1,217 cases of intestinal disease presenting symptoms of either diarrhoea or dysentery included :

484 cases of amoebic dysentery, proven either by the demonstration of *Entamoeba histolytica* in the faeces or by sigmoidoscopic examination.

408 cases of sprue.

109 cases of chronic bacillary dysentery.

AMOEBIC DYSENTERY LESIONS AS SEEN BY SIGMOIDOSCOPY.

This account has been compiled from 183 cases of amoebic dysentery, examined in routine manner, preliminary to treatment.

There are certain points in the pathology of amoebic dysentery which render it distinguishable from the bacillary disease. In about 80 per cent. of all amoebic infections of the bowel, the lesions appear first in the rectum. (Naturally the exact proportions are difficult to verify as this can be done solely by examining consecutively a very large series of cases.) In the remaining 20 per cent. the lesions are situated higher up in the bowel in the descending colon, or in the caecum beyond the range of the sigmoidoscope. The experimental studies

of WAGNER and of BIELING,* which have recently been published, tend to show that in animals experimentally infected *per os*, the first lesions appear in the rectum. Sigmoidoscopy provides an easy method of inspection of the earliest lesions of this disease. It is generally acknowledged, too, that it is by no means easy to diagnose every case of amoebic dysentery, especially in the chronic stage, by the organism in the faeces. It has happened in my practice that the cysts of *E. histolytica* may be found in the faeces for the first time after fifty consecutive faeces examinations. Concentration methods (as for instance, that of Warrington Yorke) as a means of demonstrating the cysts, when they are scanty, have not proved of great practical importance ; nor have cultural methods afforded us any superior means of demonstrating the *E. histolytica* when they are scanty in the faeces.

The lesions of amoebic dysentery, as seen in the rectum, are naturally in the main similar to those with which the pathologist is familiar in the postmortem room. These lesions are usually distinctive and easily recognizable, but they are much finer and smaller than the larger ulcers commonly found higher up in the large bowel. The fact that they are seen in the living state naturally has an influence on their appearance. At postmortem many of the finer colours and smaller haemorrhages have disappeared, so that the clinician will not gather an accurate impression of what he is likely to meet with from a study of museum specimens.

The *E. histolytica* is generally understood to be a parasite of the submucosa, in which layer it burrows and forms the characteristic flask-shaped ulcer. At first, in the human subject, the site of these submucous burrowings may be marked by small yellow elevations or papules, which, when they rupture and discharge their contents into the bowel, assume the more familiar appearance of amoebic ulcers. In early cases of amoebic infection of the rectum, these small, yellow elevations may be detected and recognized. The amoebic ulcers, when they develop, are seldom of any great size, and are usually represented by small red depressions, up to 5 mm. in diameter, usually with a haemorrhagic margin. In the immediate neighbourhood small petechial haemorrhages are scattered about in a haphazard manner. Often in more acute cases larger haemorrhages may be recognized in interstices of the mucosa or in ragged cracks.

The mucous membrane between the individual ulcers is singularly unaffected. Usually it is of a pale yellowish pink colour, and it has often been pointed out that in amoebic infections the folds of mucous membrane are more lax and reticulated than in the normal subject, in contrast to the contracted rigid and stenosed appearance commonly encountered in the chronic stage of bacillary dysentery. Very occasionally solitary amoebic ulcers of a large size, up to 1 cm. in diameter, occur in the sphincteral region or on the columns of Morgagni,

*WAGNER, O. & BIELING, R. (1935). *Beiheft z. Archiv. f. Schiffs- u. Trop. Hyg.*, xxxix (1), 1948, 1-48, 49 108.

and may be so indurated as to simulate a malignant ulcer : I have encountered three of these in my experience. However, in these cases, the amoebic nature of the lesions was verified by making microscopic preparations from the lesions and recognition of the contained amoebae. Amoebic dysentery does not, in my opinion, ever produce actual stricture of the bowel, but occasionally it may cause a localized obstruction by forming localized hyperplastic outgrowths, resembling a tumour. These hyperplastic granulomata are by no means common, but I have seen one instance where the amoebae were demonstrated in sections of such a growth removed at operation. GUNN and HOWARD* have described three such cases in which the resemblance to carcinoma was very close indeed.

In the very acute cases of amoebic dysentery which are seldom met with in tropical practice in this country, quite a distinctive form of surface infection of the mucosa with the *E. histolytica* is met with. This has the appearance of an excoriation and general destruction of the mucosa resembling that in bacillary dysentery, or the surface infection commonly encountered in the experimental disease produced in kittens. The appearance of the mucosa is then quite different and can with difficulty be distinguished from the granular mucosa of chronic bacillary dysentery, or the raw bleeding surface of ulcerative colitis. In these cases, of which I have now seen five, the amoebae may be demonstrated in large numbers in preparations made from the bleeding mucosa. These bleeding lesions extend right down the rectum to the internal sphincter, and may involve the anal margins.

In chronic amoebic dysentery, in which *E. histolytica* cysts are present in the faeces, the lesions in the rectum are not so easily detected as in the more acute stages. They are often represented by very minute depressions or pits, which can be distinguished only by using the magnifying eyepiece. Then the mucous membrane, when the light strikes on it from an angle, with the mucosa half inflated (by the bellows) appears superficially " pock marked," or as if nibbled by microscopic mice. The pittings really represent the depressions, or scars, left by the healing of the minute amoebic ulcers of the more acute stage. They are by no means easy to recognize with certainty, and this can be done solely by constant practice. It is extremely difficult, if not impossible, to demonstrate the amoebae in scrapings or preparations made from those lesions, probably because in the majority of instances, these organisms are scanty or have disappeared.

What proof have we then that these do really represent defunct amoebic lesions ? This proof has been obtained by making frequent sigmoidoscopic examinations of cases whilst undergoing treatment, when these depressions can be seen to persist at the site of healing amoebic ulcers. This " pitted " condition of the mucosa may be general or may occur in patches in the rectal ampulla, and occasionally in ring-like zones. They have to be distinguished from the apertures of the racemose anal glands which have already been described.

*GUNN, H. & HOWARD, W. J. (1931). *J. Amer. Med. Assoc.*, xcvii, 163-170.

I have found that these are the only lesions usually visible in symptomless carriers, or cyst-passers, of *E. histolytica*. However, it must be admitted that in many cyst-passer cases, the mucous membrane appears perfectly normal. I have been able to ascertain, too, that the cysts of *E. histolytica* are more numerous in the faecal ribbons adhering to the bowel mucosa, than in the faeces themselves, and this is a practical point in diagnosis. It is always worth while making scrapings from any suspected amoebic lesion in the rectum. It is not always easy to obtain enough tissue (blood and mucus) in the hollow of the Volkmann spoon for microscopic study. When the lesions are bleeding with haemorrhagic and punched-out margins, the organisms are usually abundant and occasionally are visible in strings or in masses. Sometimes, too, material is obtained containing numerous Charcot-Leyden crystals which are also suggestive of an amoebic infection of the bowel. It is, however, in the acute bleeding surface infections that amoebae can best be demonstrated in " scrape preparations."

The sigmoidoscope as a guide to treatment in amoebic dysentery.

In addition to their diagnostic uses the sigmoidoscopic appearances are most useful in assessing the value of different forms of treatment and as a gauge of the amoebicidal action of various drugs. The therapeutic diagnosis is often most valuable. When there is any doubt about the character of intestinal ulcers, and when it has not been possible to demonstrate the causative amoeba, then the rapid healing of the lesions on instituting emetine, emetine-bismuthous iodide or yatren therapy is so striking as to be absolutely diagnostic. With the emetine, emetine-bismuthous iodide and yatren treatment which I have described, the healing of the lesions is extraordinarily rapid. Within 12 days from the commencement, granulations have formed and filled up the small ulcers, the surface epithelium has covered the depressions and all that can be seen is small pink spots which are redder than the normal mucosa and which represent the site of the former ulcers. Apparently the formation of depressions, or pits, takes place at a later stage. I have also ascertained that healing commences in acute cases after the injection of 3 grains of emetine.

THE SIGMOIDOSCOPIC APPEARANCE OF CHRONIC BACILLARY DYSENTERY.

It is in the chronic stages of bacillary dysentery or in the aftermaths of the severe disease, that it is most important to make a diagnosis and to differentiate it (not always an easy matter) from chronic amoebic dysentery. The sigmoidoscope, almost alone, provides the surest method of making the diagnosis. It is almost useless to attempt to isolate the dysentery bacillus from the faeces and the microscopic examination of the excreta yields usually but little information beyond the presence of a few distorted pus cells. I have found the serum agglutination test, using a pooled emulsion of the serological races of Flexner's bacillus, a useful adjunct in arriving at a diagnosis, but it is not by any means always helpful.

The cases of chronic bacillary dysentery, as seen in practice at the Hospital for Tropical Diseases, are rarely subsequent to an acute initial attack, rather the chronic stage supervenes insidiously. The sigmoidoscopic appearances differ considerably from those described in amoebic dysentery. The bowel wall is rigid and inelastic. The lumen is always to some extent stenosed. Instrumentation is painful, whilst in amoebic dysentery, though uncomfortable, it is distinctly painless. The surface of the mucosa is granular, usually carmine or rosy red, in appearance ; owing to the formation of granulation tissue it is rough and grazed, and the chief feature is its spongy and easily traumatized character. Therefore, there is usually surface-oozing on withdrawal of the tube. It is to be noted, too, that the folds of Houston are either retracted or obliterated altogether. The inflammatory affection permeates all the layers of the bowel wall, so that the tube is rigid and the normal mucosal folds are lacking. In the less advanced stage attention should be focussed on the outline of the smaller blood vessels, which are usually visible. The changes in these small veins are as important as in ophthalmoscopy of the retina, and can be taken as an index of the inflammatory changes which have taken place in the mucosa.

The granulation tissue does not always extend throughout the rectum, but may occur in patches or ridges, sometimes as an encircling zone. It is not always easy to distinguish from similar granular stages in ulcerative colitis, but the scattered character of the lesions already referred to may be of some avail. Fibrotic scarring of the mucosal surface is sometimes seen, as is also the formation of pseudo-polypi. Partial stenosis of the bowel has been observed. (See statistics, p. 558.)

The patulous condition of the anal margins, and the atrophic appearance of the surrounding skin, as well as the wasting of the gluteal and perineal muscles, afford some additional evidence in arriving at a diagnosis in a doubtful case.

THE SIGMOIDOSCOPIC APPEARANCES OF SPRUE.

Sigmoidoscopic examination forms an additional means of arriving at a diagnosis in sprue. I am of the opinion that the pathological changes in the mucosa (whatever their true aetiology may be) extend throughout the large intestine and may, therefore, be visible in the rectum. The occurrence of these appearances, especially in fairly early cases with acute symptoms, conveys the impression that sprue is an affection of the whole of the intestinal mucosa. It is more than probable that the changes seen in the rectum and sigmoid are of a similar nature to those usually observed in the mouth and tongue. The mucous membrane is ridged and folded, and easily traumatized. It is very finely granular on the surface and in colour, cherry-pink. The intestinal mucus is sticky and adherent, whilst the typical sprue faeces—yellowish, or white in colour —can be seen pouring down the lumen of the bowel through the recto-sigmoid sphincter. Possibly the irritation of the mucous membrane is caused by an excess of fatty acids in the faeces.

It is my experience that the upper rectum in sprue always contains faeces, and they have a tendency to adhere to the bowel wall. On several occasions I have been able to make a correct diagnosis of sprue in a subacute and ill-defined case, by observing the character of the faeces seen through the sigmoido-scope—that is, when the stool as passed into the pan was by no means char-acteristic.

In the advanced stages of sprue, when atrophy has taken place, the mucosa is lax, pale yellow in colour and parchment-like. Sometimes it appears so friable that an extensive sigmoidoscopic examination is not without danger.

SIGMOIDOSCOPIC APPEARANCES IN LYMPHOGRANULOMA
(THE GENITO-ANO-RECTAL SYNDROME.)

The occurrence of stenosis and actual stricture of the bowel is a com-paratively rare event in tropical practice. By permission of my colleagues, I have made an analysis of 3,068 sigmoidoscopic examinations performed in the Hospital for Tropical Diseases during the last 16 years, and have found that evidence of stenosis and stricture was present in 0·8 per cent. of the cases. The only real strictures which were diagnosed were due to carcinoma, syphilis and lymphogranuloma. In two only, fibrous strictures 5 cm. from the anus due to lymphogranuloma inguinale were found, and in both of these the Frei-Hoffmann test was positive. The strictures were pale yellow in colour, of dense fibrous consistency and associated with scarring. In one case, actual ulceration of the mucosa and fistulae also were present.

The great rarity of this lesion in tropical practice as compared to its com-parative frequency in Paris as recorded by BENSAUDE and LAMBLING,* is a matter for comment.

The statistics obtained by this enquiry are as follows :—

DYSENTERIC CASES EXAMINED BY SIGMOIDOSCOPY.

Total, 3,068 cases.

Of this total, stenosis and stricture of the rectum occurred in 27 cases (incidence, 0·8 per cent.) diagnosed as follows :—

	Cases.
Chronic bacillary dysentery	7
Chronic amoebic dysentery (including pericolic abscess)	5
Ulcerative colitis	2
Tuberculosis of colon	2
Syphilitic stricture	1
Gonorrhoeal proctitis	1
Polyposis	1
Lymphogranuloma inguinale stricture	2
Diverticulitis	1
Carcinoma of rectum (post-dysenteric)	5

*BENSAUDE, R. & LAMBLING, A (1936). *Proc. Roy. Soc. Med.*, xxix, 1441-1449.

SIGMOIDOSCOPY IN TROPICAL PRACTICE.

BY

LT.-COL. A. G. BIGGAM, M.D., F.R.C.P., R.A.M.C.

Royal Army Medical College, Millbank, London.

TECHNIQUE.

The preparation of the patient is an important part of the procedure. When the physical condition is such that any detailed preparation is not possible, all that is necessary is to evacuate the bowel by means of a simple enema an hour or so beforehand and to ensure that as much as possible of the fluid is voided before the examination.   When the condition of the patient allows of a more thorough preparation it is best to avoid any preliminary purgation, as this may cause increased peristalsis and flooding of the sigmoidoscopic field from above. In normal circumstances, the day prior to the examination, the patient is put on a diet low in residue, and early the following morning an ordinary soap and water enema followed by an alkaline washout is given.   Unless any contra-indication exists the patient should be encouraged to move about as this will ensure more thorough evacuation of the bowel.   In the case of nervous or apprehensive persons morphia, $\frac{1}{4}$ grain, can be given half-an-hour before the examination, but this, however, is seldom necessary.

The most comfortable position for the patient, and one convenient for the operator, is on the right side with the knees drawn up and with the buttocks close to the edge of the table.   This reduces strain to a minimum, an important point in the weak and debilitated.

A special table has been designed for sigmoidoscopic examination which allows the patient to be tilted with the buttocks elevated.   This precludes the possibility of fluid welling up into the tube.   The position is, however, trying and is not suitable if the patient is seriously ill.

During the insertion of the instrument, it is usually necessary to employ gentle inflation to open up the lumen of the bowel for the passage of the tube and to expose lesions of the mucosa.   A satisfactory view can generally be

obtained, although swabbing may be necessary to clear away the fluid and solid particles. The healthy mucosa appears pink and glistening and resembles the inner side of the cheek—the depth of colour, of course, varying with the degree of anaemia of the patient. The sigmoidoscopic appearances in the different types of dysentery are usually very characteristic and frequently allow of a diagnosis to be established at once.[*]

BACILLARY DYSENTERY.

Where the patient suffering from acute bacillary dysentery has not been seen until after the first 5 days of the onset, isolation of the specific organism may not be possible. In such cases a visual examination of the bowel through the sigmoidoscope is of considerable value. The instrument need only be inserted a very short distance inside the sphincter to give a sufficiently adequate view of the mucosa for a diagnosis to be made.

The characteristic change noted in bacillary dysentery is a widespread inflammation of the mucosa, its intensity of course varying with the type of the infecting organism. In very acute cases the gut wall is oedematous, rigid, and often difficult to dilate sufficiently to allow of insertion of the instrument, which may cause considerable pain. The mucous membrane is acutely inflamed and cherry red in appearance. It bleeds readily and the lumen of the gut is usually found to contain much mucus and blood. This, by obscuring the field, may interfere considerably with the examination. Ulceration, when present, is superficial, surrounded by inflamed mucosa and is in marked contrast to the ulceration found in amoebic dysentery. In the most severe cases when extensive coagulative necrosis has occurred the mucosa is greyish green in colour, obviously dead, and in places may be seen to be exfoliated exposing the under-lying tissue. This appearance suggests a very grave prognosis.

Mild cases of bacillary dysentery show much less marked changes. The degree of inflammation is much less and is frequently limited to irregular patches mainly disposed transversely in the direction of the folds of the mucous membrane. Ulceration is infrequent and blood and mucus more scanty.

MALARIAL COLITIS.

The fact is frequently overlooked that subtertian malarial infection may, on occasion, produce acute dysenteric symptoms with blood and mucus in the stools indistinguishable clinically from bacillary dysentery. These symptoms are produced by the capillary obstruction caused by the malarial infection. Sigmoidoscopic examination has shown that in such cases there may be considerable damage to the mucosa of the lower bowel.[†] In the milder degrees, the mucous membrane shows diffuse or patchy hyperaemia similar to that found in a mild bacillary dysentery. When the clinical condition is more severe,

[*]BIGGAM, A. G. & ARAFA, M. A. (1930). The sigmoidoscope as an aid in the diagnosis of dysenteric conditions. *Trans. R. Soc. trop. Med. Hyg.*, xxiv, 187.
[†]BIGGAM, A. G. & ARAFA, M. A. (1930). Observations on cases of artificially induced subtertian malaria. *Trans. R. Soc. trop. Med. Hyg.*, xxiii, 593.

scattered haemorrhagic areas are seen in the hyperaemic bowel wall. Where the capillary blockage is still more intense, areas showing superficial necrosis can be noted. The extent of this necrosis varies from a few scattered patches to an involvement of the entire surface as far as the instrument can reach. These sigmoidoscopic changes are seldom sufficiently characteristic to enable a definite diagnosis to be made by this method alone.

AMOEBIC DYSENTERY.

The characteristic feature of this disease is the presence of discrete ulcers usually with more or less congestion of their margins, the mucous membrane between the ulcers being normal. The appearance of the crater of the ulcer varies considerably according to the amount of slough present. When the ulcer is clean and free from debris it has a depressed punched-out looking centre. Frequently, however, owing to the adherent slough, the greyish white centre appears to be flush with the surface of the bowel. On occasions the whole ulcer is completely hidden by debris and mucus. Unless, therefore, care has been taken to have the bowel well prepared and to swab away any mucus still adherent to the bowel wall, typical amoebic ulcers may be missed. Material for microscopic examination should always be obtained from the ulcer to confirm the diagnosis by the demonstration of motile *Entamoeba histolytica*.

The number and size of the ulcers varies greatly with the severity of the disease. In slight cases only very small ulcers may be present, or there may be none evident within reach of the instrument. Usually, however, even in very mild amoebic dysentery if care be taken to search the bowel just within the internal sphincter a typical ulcer can be detected and a scraping from this will reveal *E. histolytica*.

Occasionally, especially in very debilitated subjects, amoebic dysentery may present a clinical picture resembling very closely that of an acute bacillary dysentery, with almost constant diarrhoea and the passage of stools usually very offensive and consisting largely of blood and mucus. Here the laboratory may fail to demonstrate *E. histolytica*, but a sigmoidoscopic examination will reveal the true nature of the disease. In such cases, extensive destruction of the mucosa is usually present and there may only remain small islands of healthy mucous membrane in the midst of these large ulcerated areas. Blood and mucus pouring down from the intestine above usually necessitates constant swabbing to enable a satisfactory examination to be made.

On recovery from amoebic ulceration the healing process is usually complete and nothing remains to indicate the previous site of the ulcers even when the area affected has been very extensive.

Useful information can be obtained by the use of the sigmoidoscope in observing the effects of certain lines of treatment in amoebic dysentery. During a trial of rivanol* in this disease, it was possible to observe the persistence of the

*BIGGAM, A. G. & ARAFA, M. A. (1930). The effect of rivanol in the treatment of amoebic dysentery. *Lancet*, June 21st, 1335.

ulcers and to demonstrate the presence of living *E. histolytica* in them despite considerable temporary symptomatic improvement.

BILHARZIAL DYSENTERY.

This disease occurs with a definite geographical distribution. The symptoms resemble those of an amoebic dysentery, but the ova of *Schistosoma mansoni* can as a rule be demonstrated in the mucoid material from the surface of the stool. In cases of intense infestation the symptoms may be so acute as to simulate an attack of bacillary dysentery. Pathological changes are very widespread in this disease and ova have been found in almost every tissue and organ of the body.

S. haematobium usually affects the genito-urinary tract ; while *S. mansoni* and *S. japonicum* have a predilection for the intestinal wall, the ova being deposited especially in the submucosa of the lower part of the colon : the irritation caused by the presence of these ova gives rise to dysenteric symptoms.

Important changes are also frequently produced in the liver and spleen by chronic *S. mansoni* and *S. japonicum* infestations.

Sigmoidoscopic examination in this disease may be of considerable help in diagnosis as sometimes ova cannot be found in the stools even by concentration methods. The lesions are usually most extensive in the lower part of the large intestine within easy reach of the instrument, but frequently changes are found even as high as the ileum. The earliest recognisable sigmoidoscopic change is hyperaemia of the mucosa, frequently patchy in distribution. In these patches are seen small red spots scattered about in the mucosa but not elevated above the surface. This appearance is very characteristic of an early bilharzial involvement. As the disease progresses raised nodules covered by mucous membrane make their appearance ; and these increase in size to form polypoid masses some of which may eventually almost fill the lumen of the bowel. They may be sessile or pedunculated, very numerous or scanty. The surface of the polypus is generally hyperaemic and bleeds readily. Degenerative changes usually supervene and these vary from superficial necrosis to destruction of almost the entire papilloma leaving behind only its stalk. Ulceration extending into the mucosa causing punched-out ulcers is rarely seen. Small superficial abrasions are, however, sometimes observed especially in the early stages of the disease. In chronic untreated cases, all the different stages in the pathological process in the intestine can be observed in the same patient. Very occasionally massive infiltration of all the coats of the bowel occurs producing a hard nodular tumour usually situated in the sigmoid region. Obstruction of the lumen of the bowel is rare as a result of the bilharzial process and malignant changes seldom supervene. Occasionally lesions in the large intestine have been observed as the result of *S. haematobium* infestation. In these cases the papillomata are characteristically flat and non-pedunculated, features which will give an indication of their origin.

Under treatment by tartar emetic the early lesions can be observed to subside rapidly while the mucosa regains its normal appearance. This resolution, however, does not occur when polypi have already formed. In such cases, the dysenteric symptoms may persist unchanged for months or years even after a full course of treatment. Some relief has been afforded by the removal of the largest of the tumours by means of an electric cautery. More recently, we have employed Marchand's special diathermy apparatus* introduced through the sigmoidoscope. This method takes considerable time when polypi are numerous but it frequently gives very good results.

Mixed infections can also be recognised by sigmoidoscopy.

DISCUSSION.

Dr. C. C. Chesterman said he was particularly interested in the description given by Colonel BIGGAM of the type of lesion produced by terminal spined ova of *S. haematobium* in the large bowel. In the new species of schistosome in the Congo (*S. intercalatum*), which also deposited terminal spined eggs in the bowel, no such appearances had been noted by Dr. FISHER or himself. All that sigmoidoscopy showed in infestation with the latter parasite was reddening and a sand-paper appearance in the mucosa.

Dr. N. Hamilton Fairley pointed out that preparation for sigmoidoscopy involving the use of aperients, and cleansing enemata often altered the appearance of the mucosa. The surest index to inflammation was the presence of bleeding when the mucosa was swabbed. Amoebic ulcers, as seen by sigmoidoscopy, were readily identified, but amoebic granulomata and diffuse amoebic proctitis presented more difficulty. In all types of lesions vegetative amoebae were readily demonstrable in scrapings. Sigmoidoscopy was of value in determining non-tropical causes of blood and mucus in the stools, and carcinoma sometimes co-existed with either active or latent amoebiasis. In the absence of cellular exudate in the stools sigmoidoscopy rarely yielded positive findings of tropical interest. In his opinion bacillary dysentery was almost invariably a self-limited disease which killed or cured, and examples of so-called chronic bacillary dysentery among prisoners of war and inmates of gaols in India probably originated from repeated reinfections over long periods.

Dr. Goldberger said that proctosigmoidoscopy should be practised as a routine procedure by every physician investigating bowel trouble. He then described his special instrument, which by means of a telescope gave a magnification of some ten diameters. It was particularly suited for strictures, and could be passed as far as 35 cm. up the bowel. By attaching a tube and using water

*BIGGAM, A. G., HASHIM, M. & GHALIOUNGNI, P. (1934). Treatment of papilloma by diathermy. *Trans. R. Soc. trop. Med. Hyg.*, xxvii, 409.

pressure it was possible to cleanse the bowel of blood and mucous exudate during the examination.

Dr. Manson-Bahr, in reply, said that definite puckering of the bowel did sometimes follow the healing of extensive amoebic ulcerations. That was the only point on which he was at variance with Colonel BIGGAM.

In regard to the remarks of Dr. HAMILTON FAIRLEY, he agreed that recurrent bacillary infection and possibly superadded amoebic infection also were responsible for the furrowed and scarred bowels seen in Turkish prisoners of war. The presence of agglutinin in the blood was the only way he knew of distinguishing chronic bacillary dysentery from ulcerative colitis.

The meeting was preceded at 7.45 by Demonstrations of specimens, pictures, charts, etc., arranged by Dr. MANSON-BAHR and by Colonel BIGGAM.

COMMUNICATIONS.

A CASE OF SEVERE SUBTERTIAN MALARIA WITH RECOVERY.

BY

J. W. FIELD, M.D.*

Institute for Medical Research, Federated Malay States.

The following records of a case of severe subtertian malaria treated in the General Hospital, Kuala Lumpur, Federated Malay States, in the course of a clinical investigation into the optimal dosage of the new soluble atebrin, presents a number of clinical and parasitological features which are possibly of general interest.

" R," a male Tamil, 30 years of age, was admitted to hospital for fever on the 7th June, 1936. He stated that he had arrived in Kuala Lumpur from Penang 4 days before and had first felt ill on the journey. He had resided in Penang for 6 years where his only illness had been an attack of pneumonia 2 years previously. He was fairly well nourished and his physical condition was good. His weight was 101 lbs., a figure not significantly below the average of his race. Examination of the blood revealed a subtertian infection of moderate severity.

On 8th June, the patient was given an intramuscular injection of 0·25 gramme of atebrin-musonate. The trophozoite count in the peripheral blood was then 64,000 per c.mm.

On 9th June, the injection was repeated. Meanwhile the count had increased to 250,000 per c.mm.

On 10th June, the temperature had fallen to normal and the patient appeared to be somewhat better. The parasite count was still high but showed a definite fall and the favourable course we had previously seen in similar cases treated at the same dosage was anticipated.

On the morning of the 11th, the condition of the patient was rather less satisfactory. There was high fever with its attendant discomforts but little clinical evidence of undue severity of infection. Thin blood films were examined as a routine precaution, for the staining and examination of thick films by the technique we employ entails a delay of nearly 24 hours, and, to our surprise,

the infection appeared many times more intense than at the beginning of treatment. Thin films showed large numbers of segmenting parasites and from twenty to fifty parasites per field. In thick films prepared by SINTON's fowl-corpuscle method parasites were far too numerous to be counted with accuracy. A relative count of infected cells and of parasites made from a thin film was as follows :—

<div align="center">TABLE I.</div>

Total R.B.C. Counted.	Parasites.	Infected Cells.	Single Infection of Cell.	Double Infection of Cell.	Triple Infection of Cell.	Quadruple Infection of Cell.
5,000	896	797	682	93	8	1

In one cell—not included in the count—five ring forms were identifiable.*

The Plate illustrates the intensity of infection. The two fields represented are reproduced from water colour drawings made for us by Mr. CHARTON. Many of the ring forms are seen to be at a fairly advanced stage of development. Three parasites in the thin film field and no less than ten in the thick film field show relatively advanced schizogony.

It was not possible to make an accurate count of the parasites by the standard fowl-cell technique. A red cell count made on the 11th June was 3,700,000 and as the proportion of parasites to total red cells in a thin film was 17·9 : 100, the total parasite count was in the region of 660,000 per c.mm.

A point of clinical interest was the extraordinary discordance between the clinical and parasitological findings. When blood films taken on the morning

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*The heaviest subtertian infection we have been able to trace in departmental records covering the treatment of some thousands of cases of acute malaria over a period of nearly 10 years, occurred in a young Chinese who was admitted to the same hospital in June, 1935, and died within a few hours. The relative parasite to red cell count in this case is given for purposes of comparison.

Total R.B.C. Counted.	Parasites.	Infected Cells.	Single Infection of Cell.	Double Infection of Cell.	Triple Infection of Cell.	Quadruple Infection of Cell.	Quintuple Infection of Cell.
5,000	2,470	1,536	1,463	363	77	10	2

In thin films taken from this case on the day of death the presence of six rings in a single red cell was noted on several occasions.

Heavier infections have been recorded. CHOPRA and SEN (1932), for instance, record a case in which 67 per cent. of the red cells were infected, the parasite count being estimated as 2,800,000 per c.mm. (*Indian med. Gaz.*, lxvii, 12, 680).
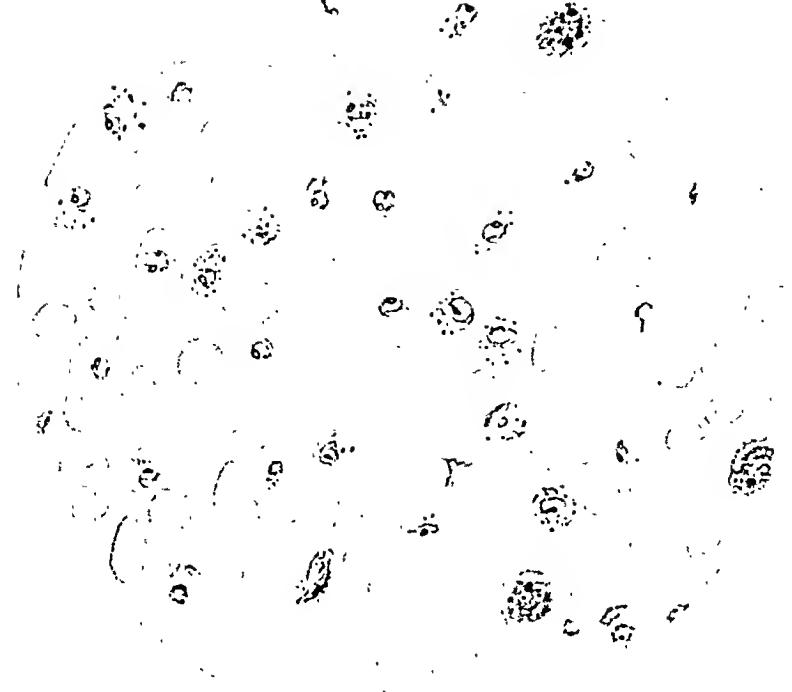
J. R. Charton

Water colour drawing of a field from a thin film taken at noon on 11.6.36. (*Standard Zeiss microscope, 1/12" fluorite objective, Bitukni binocular attachment with × 7 eyepieces*).
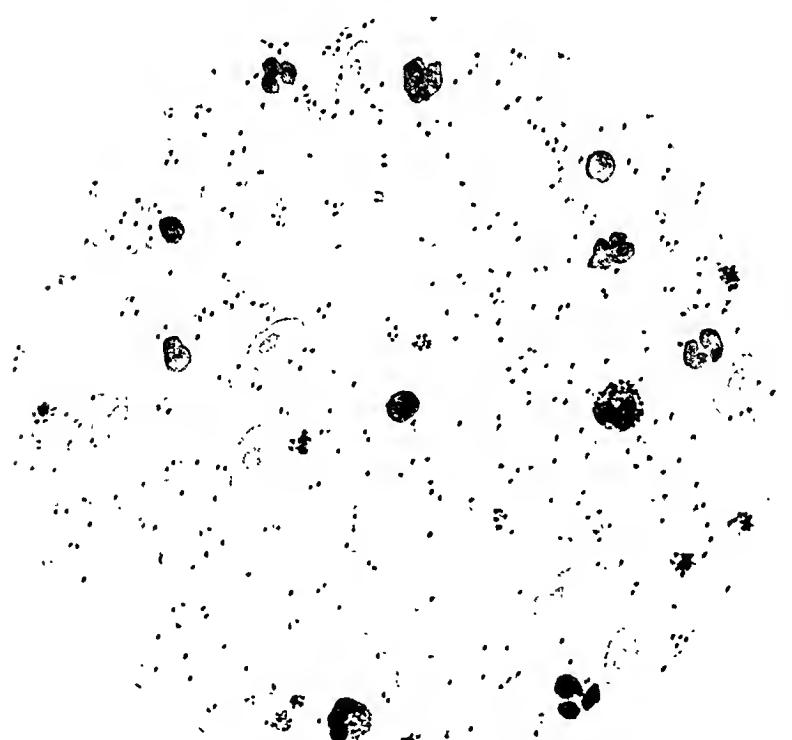
J. R. Charton.

Water colour drawing of a field from the thin edge of a fowl-corpuscle film taken at noon on 11.6.36. (*Standard Zeiss microscope, 1/12" fluorite objective, Bitukni binocular attachment with . 7 eyepieces*).

COMMENT.

It is not suggested that this case is unique.   It is of interest in so far as recovery from a subtertian infection of the intensity described is a rare phenomenon in the Kuala Lumpur district of the Malay States.   How far this finding is generally true in other parts of the world we have been unable to ascertain. It is for this reason that the facts of the case have been placed on record.

SUMMARY.

1. Brief clinical and parasitological details of a case which recovered from a malarial infection of unusual severity are described.

2. It is noted that little information seems to be available regarding the highest degree of infestation with the parasites of human malaria which is consistent with life.

A NOTE ON PROGNOSIS IN RELATION TO PARASITE COUNTS IN ACUTE SUBTERTIAN MALARIA.

BY

J. W. FIELD, M.D.

AND

J. C. NIVEN, M.B., CH.B.

Institute for Medical Research, Federated Malay States.

It is universally recognized that prognosis in acute subtertian malaria bears a general relationship to the concentration of parasites in the peripheral blood though it is usually understood that this relationship is not a very close one.   Blood films from cases which, on clinical grounds, appear to be severe, occasionally show relatively few parasites and it is thus sometimes suggested that prognosis based on parasitological findings is unreliable.

An analysis was recently made of over 5,000 parasites counts from 750 cases of acute subtertian malaria following a suggestion that it might be possible to define the prognostic significance of blood examination in somewhat more precise

terms. The findings have seemed to us to be of sufficient interest to justify a brief note on the subject.

Over the past few years one of the activities of the Malaria Research Division of the Institute for Medical Research, Federated Malay States, has been the evaluation of new malarial remedies by clinical tests on cases of acute malaria admitted to the Government hospitals adjoining the Institute. Approximately 2,000 cases of acute malaria are admitted annually to this hospital group from the surrounding districts where malaria smoulders throughout the year. From this source of clinical material cases are selected for experimental observation in such a way as to ensure, so far as possible, the elimination of those likely to show a misleading response to treatment with test remedies. Selection is limited, for instance, to individuals :—

(*a*) Who have had no recent malarial treatment.

(*b*) Whose blood shows the presence of a single species of parasite in significant numbers (500 per c.mm.).

(*c*) Who have fever at the time of examination.

These standards entail the elimination of many mild infections but otherwise there is no selection of cases in relation to clinical severity of infection. Treatment is subject to the parasitological control appropriate to the evaluation of remedies under test. Trophozoite and gametocyte counts by SINTON's fowl-cell method are made daily during a minimum treatment period of 7 days, counts of over a quarter of a million per c.mm. being checked as a rule by haemocytometer counts of red cells and relative parasite to red cell counts in thin films.

Most of the cases are male adults of Chinese, Indian or Malay race.

TABLE I.

DEATH RATE IN RELATION TO THE PARASITE COUNT ON THE FIRST DAY OF TREATMENT IN 750 CASES OF ACUTE SUBTERTIAN MALARIA.

	Trophozoite Counts per c.mm. of Blood on First Day of Treatment.					
	Under 5,000.	5,000 to 25,000.	25,000 to 100,000.	100,000 to 250,000.	250,000 to 500,000.	Over 500,000.
Cases	209	261	205	57	10	8
Deaths	1	0	1	4	2	5
Death rate per cent.	0·5	0	0·5	7	20	63

The parasite counts made on the first day of treatment in a series of 750 cases of acute subtertian malaria selected and observed under the conditions defined are summarized in Table I. Most of these cases were treated during the year 1935 and 1936 when the infections prevalent in the Kuala Lumpur district were of normal incidence and severity.

The daily trophozoite counts from the thirteen fatal cases are summarized in Table II.

TABLE II.

DAILY TROPHOZOITE COUNTS IN THIRTEEN FATAL CASES OF ACUTE SUBTERTIAN MALARIA.

| Case Number. | Trophozoite Counts per c.mm. of Blood. | | | | | | | Remarks. |
| | Day. | | | | | | | |
	1st.	2nd.	3rd.	4th.	5th.	6th.	7th.	
TST 73/32	2,300	55,000	100	0	0	0	0†	Basal pneumonia found at post-mortem examination.
AMST 2/35	35,000†							Condition on admission, poor. Haemoglobin 25 per cent.
AST 26/35	108,000	Over 500,000	†					
QST 24/32	178,000	40,000	200	0	0	0	0	Died 12th day during an attack of Shiga dysentery.
QST 68/35	180,000†							
AST 45/35	228,000	†						
QST 167/36	300,000	148,000†						
AST 44/35	450,000	370,000	228,000	48,000	3,000	300	0	Died 22nd day. No parasites found. Cause of death uncertain.
AMST 35/35	Over 500,000†							
QST 197/36	Over 500,000	220,000	28,000†					Mentally clouded on morning of 3rd day. Comatose in afternoon. Died in evening.
AST 73/35	650,000†							
AMST 5/35	700,000	272,000	†					
QST 209/36	540,000	50,000	7,000					Sudden coma and death on 3rd day.

One parasite per thin film field corresponds roughly to a count of from 10,000 to 20,000 per c.mm. according to magnification of the optical system, the size of the microscopic field, the spread of the red cells, etc.

Twelve cases recovered from infections in which a quarter of a million or more parasites per c.mm. were present in the peripheral blood at some time during treatment. The daily counts on these cases were as follows :—

TABLE III.

DAILY PARASITE COUNTS IN TWELVE CASES OF ACUTE SUBTERTIAN MALARIA RECOVERING FROM INFECTIONS ASSOCIATED WITH COUNTS OF 250,000 OR MORE PARASITES PER C.MM. OF PERIPHERAL BLOOD.

Case Number.	Daily Trophozoite Counts per c.mm. of Blood during Treatment.						
	1st Day.	2nd Day.	3rd Day.	4th Day.	5th Day.	6th Day.	7th Day.
AST 25/35	120,000	348,000	94,000	3,000	100	0	0
AST 56/35	300,000	4,800	54,000	3,200	100	0	0
AMST 173/36	360,000	100,000	14,000	55,000	2,000	0	0
AMST 182/36	64,000	250,000	220,000	662,000	414,000	170,000	6,000
AMST 201/36	250,000	156,000	101,000	8,000	10,000	400	600
QST 3/35	Over 500,000	Over 500,000	7,600	100	0	0	0
QST 40/35	460,000	17,000	300	2,000	100	0	0
QST 60/35	450,000	60,000	200	200	0	0	0
QST 124/35	540,000	120,000	87,000	3,000	200	800	1,000
QST 128/35	300,000	60,000	42,000	8,000	100	0	0
QST 199/36	280,000	250,000	3,600	100	0	0	0
TST 70/32	370,000	4,400	103,000	1,200	100	0	0

COMMENT.

An attempt is made by an analysis of daily trophozoite counts from a series of cases of acute subtertian malaria, treated for the most part during the past 2 years, to give statistical definition to the prevailing view that prognosis in subtertian malaria is related to the number of parasites circulating in the peripheral blood. It is noted that whereas there were only two deaths among 675 cases with counts of less than 100,000 per c.mm. on the first day of treatment, there were eleven deaths among seventy-five cases with initial counts over this figure, and no less than five deaths among eight cases with counts of over 500,000.

It should perhaps be emphasized that this close relationship between parasite concentration and prognosis was found only where no malarial drug had been given immediately prior to the examination of the blood. Parasite counts made on the second and third day of treatment were a less reliable criterion of severity. Cases QST 197 and 209, for instance, died on the third day of treatment at a time when the trophozoite counts were 28,000 and 7,000 per c.mm. of blood, whereas the initial counts in both cases were over 500,000.

Death was due in these two cases to the localization of parasites in the cerebral capillaries. On several occasions there was a marked drop in the parasite count on the second day of treatment followed by a rise on the third day (e.g., Cases AST 56, TST 70), and while it was generally true that parasite counts made at any time during treatment provided useful information of progress, it was evident, from the occasional occurrence of anomalous findings, that the prognostic indications afforded by blood examination could be accepted with confidence only on the first day of treatment.

The counts recorded are also of interest in relation to the limit of tolerance of Asiatic adults for the strains of *Plasmodium falciparum* prevalent in the Malay States. Recovery from infections associated with a parasite concentration in the peripheral blood of over a quarter of a million per c.mm. is apparently not uncommon; in the present series more of such cases recovered than died. With counts of over half a million recovery is still possible, three such cases recovered out of eight treated. The highest verified count associated with recovery was 662,000 per c.mm. (Case AMST 182). We are thus disposed to assume, pending the collection of more extensive data, that the greatest concentration of *P. falciparum* in the peripheral blood consistant with survival is in the region of three quarters of a million per c.mm.

SUMMARY.

An analysis is made of parasite counts made on the first day of treatment in a series of 750 cases of acute subtertian malaria. The analysis affords data of the parasitological intensity of the subtertian infections prevalent in the Kuala Lumpur district of the Malay States during 2 years of normal malarial incidence and severity, and suggests :—

(*a*) That parasite counts made on the first day of treatment are of considerable prognostic reliability.

(*b*) That the counts made during the course of treatment are a useful guide to progress but are open to occasional fallacy.

(*c*) That the extreme limit of tolerance of Asiatic adults for the local strains of *P. falciparum* is probably in the region of three quarters of a million parasites per c.mm. of peripheral blood.

PULMONARY BILHARZIASIS.

BY

H. B. DAY, *M.C.*, M.D., F.R.C.P.*

From Kasr-el-Aini Hospital, Cairo.

Though BELLELI of Alexandria reported the presence of bilharzia ova in the lungs in 1885, the frequency and importance of this complication of bilharziasis has hardly yet obtained recognition.   At autopsies in Witwatersrand on natives dying from various diseases, TURNER (1909) found that the lungs very frequently contained numbers of ova, the condition occurring as a complication of urinary bilharziasis.   Since ova were often numerous and were present in a larger proportion (64 per cent.) of patients dying of respiratory diseases than from other causes (33 per cent.), TURNER was of the opinion that the bilharzial lesions might predispose the lungs to bacterial infections.

Within recent years several clinical reports of cases of pulmonary bilharziasis have appeared.   SUAREZ (1930) described cases from Porto Rico with symptoms of asthma, while MAINZER (1935) reported five Egyptian cases that simulated pulmonary tuberculosis.   The important cardiac complications that may arise in advanced pulmonary bilharziasis were described by ASMY of Cairo in 1932.   He recorded two remarkable cases in which extensive lung deposits had caused such vascular obstruction that great dilatation of the pulmonary artery ensued with relative incompetence of its valves.   One patient died from right heart failure and the diagnosis was established at autopsy ;   the other showed exactly similar symptoms but recovered sufficiently to take his discharge from hospital.   CLARK and GRAEF (1935) have since published a case from America in which pulmonary bilharziasis led to death from heart failure.   At autopsy an extensive arteritis in the lungs with atheroma of the pulmonary artery was found ;   there was no incompetence of its valves.

The purpose of the present communication is to describe three cases of pulmonary bilharziasis that came under my care, and to give a brief summary of our somewhat scanty knowledge of this condition.

*My thanks are due to Prof. B. SHAW for the postmortem notes and microscopic sections illustrated in this paper.

Case Reports.

Case 1.

A boy aged 10 years was admitted for some ill-health, slight cough and wasting. There was slight irregular fever, and occasional crepitations were heard over the lower parts of both lungs. A radiograph of the chest showed no appearances suggestive of tuberculosis or broncho-pneumonia ; there was no enlargement of the hilar glands. The urine was normal. The liver and spleen were enlarged and firm ; a sigmoidoscopic examination showed that a *Schistosoma mansoni* infestation was present. Blood : Leucocyte count 10,000, eosinophils 50 per cent.

The diagnosis of a general infestation by *S. mansoni* in an early stage was made and a prolonged course of tartar emetic given. · The fever subsided, the liver and spleen became reduced in size, the signs in the lungs disappeared and the boy rapidly put on weight. He was discharged in excellent health.

Case 2.

An undersized and wasted young man of 20 was admitted to Kasr-el-Aini Hospital for swelling of the abdomen that had followed on an attack of fever two months before.

The patient had a pellagric rash and was very anaemic. The abdomen was much distended by ascites ; after tapping, the spleen was felt to be hardened, projecting one finger breadth below the costal margin ; the liver could not be palpated. The stools contained *Ankylostoma, Ascaris* and *S. mansoni* ova. Blood examination : Haemoglobin 35 per cent. ; red corpuscles, 1,840,000 ; leucocytes, 6,800 with 3 per cent. eosinophils. The urine was normal ; Wassermann negative.

He was treated with Blaud's pills, marmite and injections of novurite ; later with anthelmintics and tartar emetic in reduced doses. The ascites subsided and on palpation of the abdomen matted coils of intestine could be felt about the centre, suggestive of tubercular peritonitis. Five weeks after admission the patient succumbed to an acute bacillary dysentery (Flexner type).

Postmortem Report.—The abdominal cavity contained some rather turbid fluid. The small intestines were matted together on their anterior aspect by adhesions. Here the peritoneal covering was studded with firm, whitish nodules, often agglomerated into patches of larger size. Scrapings showed them to contain *S. mansoni* ova. The parietal peritoneum and the under surface of the intestines were free. Numbers of female bilharzial worms unaccompanied by males were found in the splenic vein—a very unusual condition ; some females and a few males were also present in the portal vein.

Both the small and large intestine were extensively thickened by bilharzial infiltration ; there was no formation of papillomata. The colon and rectum showed acute congestion and recent ulceration of the mucosa. The mesenteric

and abdominal lymph nodes were infiltrated with bilharziomata; in som
the normal structure had been entirely replaced. The spleen was moderate
enlarged with thickened capsule; the liver was slightly nodular with distin
thickening of the portal tracts from fibrosis, and the pancreas was also fibroti
No lesions were found in the urinary bladder. The heart was not affected
the bone marrow of the femur was red and hyperplastic all along the shaf

The lungs showed numerous greyish white and firm nodules, 1 to 2 mn
in diameter all over the cut surface, more abundant in the lower lobes. Micro
scopically, bilharziomata of varying sizes embedding ova accounted for thes
nodules, but the most striking picture was afforded by dilated arteries packe
with coupled worms. These are reproduced in Figs. 1, 2 and 3. Fig. 4 show
the great thickening of a small artery with the formation of a giant cell in i
wall, provoked by the presence of an ovum which has almost been removed.

The liver revealed the presence of ova in fibrotic infiltrations, while ther
was considerable pigmentation, chiefly in Küpfer's cells. The pancreas was muc
fibrosed with many bilharziomata. Sections of the thickened intestines showe
numerous ova deposited in submucous and subperitoneal infiltrations wit
coupled worms in the veins.

This case is remarkable on account of the extraordinary infestation of th
lungs with worms, apparently a pure *S. mansoni* infection. The only other cas
recorded is by SYMMERS (1905) who found a coupled pair in the blood from th
left lung, also in a case of intestinal bilharziasis.

Case 3.

A man, aged about 35, was admitted for congestive heart failure. H
had had a large spleen removed 2 years previously and had suffered from short
ness of breath for a few months.

On examination, there was a diffuse precordial pulsation extending up t
the third left space, but though the heart was enlarged no definite murmur
could be heard. Radiography revealed an enlargement of the heart involvin
the right ventricle and the conus; no special pulmonary lesions other than
congestion were visible. The blood pressure was not raised; the Wassermann
reaction was negative. The liver was hard and cirrhotic but not enlarged. Th
patient died a few days after admission.

Postmortem Report.—The right ventricle was much dilated; the hear
muscle and valves appeared healthy. The pulmonary artery was dilated and
atheromatous; the aorta was unaffected. The lungs showed some pleura
adhesions and numerous bilharziomata on section. The liver was of norma
size, but nodular and densely fibrosed; many bilharzial worms, male and femal
were found in the portal vein. There was a granular infiltration of the recta
mucosa in which *S. mansoni* ova were present. The pancreas was also affected
in one vein a worm was found.

This case is similar to that reported by CLARK and GRAEF (1935).

PARASITOLOGY.

While ova are commonly found in the lungs at autopsies on cases of urinary bilharziasis (*S. haematobium*), it is remarkable that all the cases of pulmonary bilharziasis reported as showing clinical symptoms have been instances of *S. mansoni* infestation. This singularity is somewhat surprising, for it is usual to find *S. haematobium* responsible for aberrant bilharzial lesions in the body. As this parasite commonly reaches veins which drain into the systemic circulation, any ova that escape fixation locally may readily be carried through the right heart to the lungs. In intestinal schistosomiasis, irrespective of the species responsible, ova which escape fixation in the tissues are apt to be carried in the portal stream to the liver. Owing to the reaction they excite it is most improbable that they can usually traverse the liver capillaries to reach the lungs. A few from the rectum may enter the systemic veins, but this route seems insufficient to account for a serious pulmonary involvement in patients who show few or no signs of rectal bilharziasis.

It is doubtless significant that the cases reported here and elsewhere in which pulmonary or cardiac symptoms have arisen, have been associated with splenomegaly and hepatic cirrhosis rather than with evident intestinal disease.

The gradual obstruction of the main portal channels in the liver from bilharzial cirrhosis leads to an opening up of various anastomotic channels. There is a tendency for the venules under the capsule of the liver to become dilated and worms may be found close to the surface. Through these and other communications (retroperitoneal, etc.) ova and even worms may reach the lungs without having to pass through normal liver tissue or to travel by the inferior haemorrhoidal plexus. At present there is not enough evidence to determine the mode of travel ; we need more information on the frequency and severity of the lung affections that may complicate the various forms of schistosomiasis.

LAMPE (1927) has discussed the possibility that cercariae might be able to develop into full grown parasites in the lungs.

PATHOLOGY.

Owing to the tenuity of the alveolar walls, a considerable proportion of the ova that reach the pulmonary capillaries may readily escape into the air spaces : this can be seen in Fig. 5. The main lesions in the lung resemble those of bilharzia elsewhere in the formation of bilharzial granulomata and ultimately of fibrous nodules around ova in the alveolar septa and in the walls of the vessels. The vessels affected are arterial and the result is a constricting periarteritis that may be widespread in the lungs. The pulmonary circulation may be gravely impeded in severe cases so that the pressure in the pulmonary artery is raised. This strain causes hypertrophy of the right ventricle and early degenerative changes in the wall of the pulmonary artery, shown by atheroma and dilatation. The condition may proceed to relative incompetence of the pulmonary valves

Fig. 1.—Section of lung showing scattered bilharziomata and coupled worms in two arteries.

Fig. 2.—Section of lung showing fibrosis and vessel occupied by male and female worm.
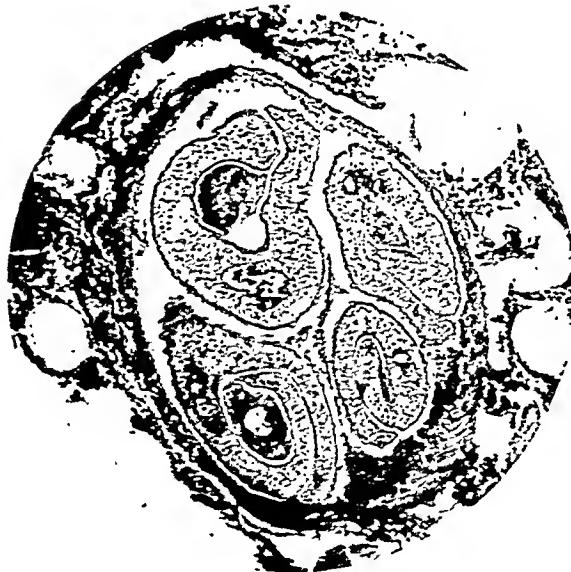
Fig. 3.—Section of lung showing dilated artery packed with worms.
The coarse tubercules on the cuticle are characteristic of the *mansoni* species of bilharzia.
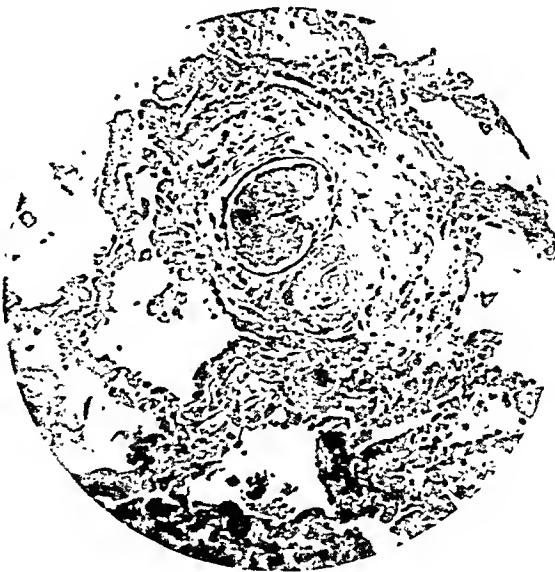
Fig. 4.—Section of lung showing arteriole with great thickening of its wall.
The lumen is occupied by blood clot and beneath the endothelium is a giant cell enclosing the remains of a bilharzia ovum.

Fig. 5.—Section of lung, showing bilharzia ova in walls of alveolus and escaping into lumen.
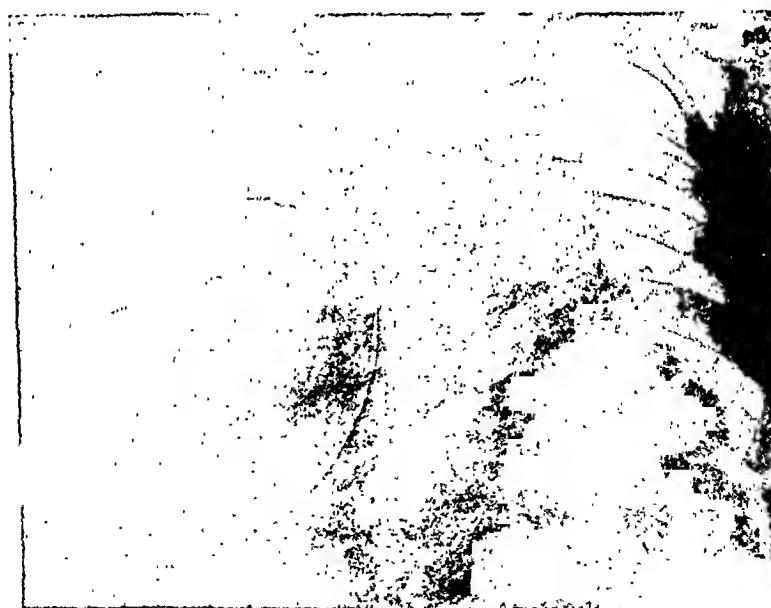
Fig. 6.—Radiograph of chest, showing aneurismal dilatation of the pulmonary artery.
Note circumscribed shadows in lungs at bases (especially right) suggestive of calcified bilharziomata.

before actual cardiac failure supervenes. Apart from cardio-vascular complications, pulmonary bilharziasis may predispose to local infections. The fibrosis of the lung and the irritation set up by the escape of ova may predispose to tuberculosis and to chronic bronchitis. KOPPISCH (1932) has published a case of pulmonary carcinoma associated with bilharziasis due to *S. mansoni* infestation.

SYMPTOMS AND SIGNS.

Cases of pulmonary bilharziasis fall into three main clinical groups—latent, pulmonary and cardio-pulmonary, according to the stage and extent of the disease.

(1) *Latent.*—The lesions in the lungs are slight and insufficient to produce symptoms during life.

(2) *Pulmonary.*—In this group the lesions are numerous enough to cause symptoms of chest trouble. Some patients after a heavy invasion by cercariae may develop a cough with fever in the initial stage, as appears to happen more commonly in the Far Eastern type (*S. japonicum*). At a later stage the presence of numerous ova in the lungs may cause cough with some sputum. When this occurs as a complication of hepatic disease, the associated wasting and irregular fever will closely resemble the clinical picture of pulmonary tuberculosis.

The physical signs are slight, merely some crepitations over the lower portions of one or both lungs. Signs are seldom found near the apices and sputum examinations are negative for tubercle bacilli. Radiographs are necessary for diagnosis; they show an absence of cotton-wool areas of infiltration and other indications of local tuberculosis, but may reveal small rounded shadows suggestive of miliary tuberculosis that are due to larger bilharzial deposits.

As in Case 1 of this series and the patients described by MAINZER, the condition generally affects young persons and is preceded by enlargement of the spleen when due to *mansoni* disease. The concurrent enlargement of the liver is a great help in diagnosis; it may be hard and nodular from cirrhosis when the disease has lasted some time.

Blood examination usually shows a high eosinophilia, ranging from 18 to 67 per cent., sufficient to cause some increase in the total number of leucocytes.

It is probable that a systematic radiological examination of patients suffering from the usual manifestations of bilharziasis would reveal a considerable proportion with pulmonary lesions that either pass unrecognized or are attributed to bronchitis.

(3) *Cardio-pulmonary.*—In the most advanced and extensive form of pulmonary bilharziasis the patient may only come under observation when heart failure occurs from progressive interference with the pulmonary circulation. This complication, associated with an advanced stage of bilharzial cirrhosis and splenomegaly, has been found in patients older than those in the second group. Bilharzial disease of the intestine and bladder may also be present, but the blood seldom shows a distinctive eosinophilia.

The most prominent symptoms and signs are those of congestive heart failure. There are dyspnoea, cough, cyanosis, engorged veins in the neck with ascites and oedema of the legs. Disturbance of the cardiac rhythm may be present.

Examination of the heart has shown some displacement of the apex outwards with evident epigastric pulsation. The area of cardiac dulness is increased on both sides and extends up to the third interspace. There may be no definite abnormality of the sounds at the apex, but over the pulmonary area a harsh systolic murmur with a palpable thrill may be present, followed by a much accentuated second sound. Should the pulmonary valves become incompetent, a diastolic murmur is heard, propagated downwards. This murmur is usually louder and harsher than an aortic regurgitant murmur ; the blood pressure readings (systolic and diastolic) are within normal limits.

Radiographs reveal an enlargement of the right ventricle, as in the familiar mitral configuration, but with a striking extension of the conus shadow upwards, indicating dilatation of the pulmonary artery, while there is no bulge below of the left auricle. (See Fig. 6 taken from a case shown by Dr. MARIE that I had the opportunity of seeing.) The lungs show the appearance of congestion with small defined shadows due to fibroid bilharziomata.

DIAGNOSIS.

In all the cases hitherto recorded of pulmonary bilharziasis, with and without heart failure, an enlarged spleen has been present. This enlargement has been associated with evidence of bilharzial cirrhosis in an early or late stage in every case seen at hospital in Cairo.

Hence, if a patient with chest trouble has an enlarged spleen which appears or can be proved to be of bilharzial origin, the possibility of pulmonary bilharziasis must be borne in mind. Bilharzial disease confined to the bladder or intestine, so far as we know at present, appears less likely to cause serious lung complications.

In young persons the general signs of wasting and irregular fever associated with a cough will naturally excite suspicion of pulmonary tuberculosis. The absence of bacilli from the sputum and of the ordinary physical and radiological signs refutes this diagnosis. In most patients the blood examination—suggested by the splenomegaly—reveals a high eosinophilia. A careful search for ova in the dejecta and an examination of the mucosa of the lower intestine and rectum by means of the sigmoidoscope will usually give a positive diagnosis. Failing this, specific tests, intradermal and serological, with bilharzial antigen are necessary.

The radiographs of some patients, as described by SUAREZ, may be suggestive of miliary tuberculosis. But, as he points out, this affection as seen in Egypt, is accompanied by high fever and runs a rapid course.

In some patients, particularly at a later stage, the symptoms and signs are

those of chronic bronchitis, possibly with some fibrosis or emphysema, and the radiographs are in conformity with this diagnosis. Although splenomegaly and cirrhosis are present, there is rarely a high eosinophilia at this more advanced stage and the nature of the pulmonary affection is doubtful.

When sputum is present in sufficient quantity, it might be possible to examine the centrifuged deposit after antiformin treatment in a search for ova. In any case the existence of active bilharzial disease requires investigation and any of the following may be regarded as positive indications :—

(*a*) An eosinophilia of 4 per cent. or more, other parasites being absent.

(*b*) The demonstration of living ova in the dejecta or in scrapings from the bowel mucosa.

(*c*) The presence of even a single papilloma on sigmoidoscopic examination.

(*d*) Definitely positive tests with bilharzial antigen.

Should a full course of specific treatment for bilharziasis cause a definite amelioration of the pulmonary condition, this may be regarded as confirmatory evidence, but not as a proof, of its bilharzial origin.

Patients with heart failure complicating bilharzial cirrhosis should be examined as far as practicable in the same way for evidence of active parasitic disease.

In these patients the presence of pulmonary arterial disease may be shown by the physical signs and actual dilatation of the main artery as seen in radiographs of advanced cases. The appearance of a grossly dilated pulmonary artery is shown in Fig. 6 while reference may be made to Fig. 1, p. 346 (No. 3) in this volume for comparison to show the general cardiac dilatation (involving the conus) in beri-beri. It is necessary that the Wassermann reaction be negative to distinguish cases of probable bilharzial origin from the usual syphilitic type of Ayerza's disease.

When there are no distinctive signs of pulmonary arterial disease the diagnosis is beset with considerable difficulty, and its discussion would range too widely to be appropriate here. But the point to be emphasised is that cardiac failure of the congestive type in cases of bilharzial cirrhosis may be dependent on pulmonary bilharziasis.

TREATMENT.

When there is evidence that a patient's symptoms may be due to active bilharziasis, there are few contra-indications to specific treatment. The presence of a cough, possibly dependent on pulmonary involvement, in a case of splenomegaly, should be no bar. If fever be present, there is seldom need to wait, for if it be due to bilharzial infection it will quickly subside on specific treatment, and thus confirm the diagnosis. When the patient is in poor condition it is well to give reduced doses, but to prolong the course accordingly. I have the impression

that *S. mansoni* is more resistant than *S. haematobium* and requires a longer course of treatment.

Cardiac failure is a very definite contra-indication to antimony, and treatment should be on the usual lines. That it may arise as a complication of an extension of bilharzial disease to the lungs is an added proof of the necessity of treating bilharzial cirrhosis (splenomegaly) by a very full specific course in the earlier stages.

SUMMARY.

Bilharziasis of the lungs may give rise to symptoms and signs that make a clinical diagnosis possible.

This condition has appeared as a complication of bilharzial cirrhosis with splenomegaly due to *S. mansoni*.

In one case here reported there was an extensive infestation of the pulmonary vessels by worms.

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THE PATHOGENISIS OF ENDEMIC (EGYPTIAN) SPLENOMEGALY.

BY

DR. ANIS BEY ONSY, D.T.M. & H. (LOND.).

Chief Pathologist, Ministry of Health Laboratories, Cairo, Egypt.

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Endemic Egyptian splenomegaly is a disease which results from a peculiar and recurrent form of inflammation caused by the repeated invasion of the splenic pulp during a long period of time by the ova of one or both species of bilharzia worm (*Schistosoma haematobium* and *S. mansoni*).

The most characteristic features of this form of inflammation are the remarkable phagocytic activities and other phenomena of resistance and body defence displayed by the macrophages of the spleen, i.e., the mobile and fixed endothelial cells.

Although bilharzial changes in the liver in their several manifestations are invariably met with (e.g., the mononuclear and the endothelial cell proliferations and also the eosinophylic cell infiltration seen in the active or early

stages and the diffuse or periportal cirrhosis noticed in the later stages) and although other manifestations of bilharzial infection in the urinary or intestinal tract or in any other abdominal organ may or may not be present, bilharzial splenomegaly does not in any way depend on the presence or on the severity of such changes, but is an independent entity.

The distribution of bilharzial splenomegaly follows very closely that of the *Planorbis* and *Bulinus* snails (the intermediate hosts of *S. mansoni* and *S. haematobium*) and the disease is, therefore, endemic all over Egypt and the Nile Valley. Like other bilharzial lesions, it is common and widespread in the lowlying districts of Egypt, particularly in the northern provinces of the Nile Delta where drains and canals are numerous and in which both kinds of infective snails are found in great numbers all the year round.

In Upper Egypt however the disease is far less common, as the basin system of irrigation is still employed; and although the snails abound during high Nile when vast areas of land are flooded, they die in enormous numbers when the land dries up during low Nile.

Whereas *S. haematobium* is found in abundance throughout Egypt, *S. mansoni* only occurs in Lower Egypt. During the past 4 years, some 1,352 enlarged spleens removed by operation have been examined from Lower Egypt, while only 48 cases have been examined from Upper Egypt. Endemic splenomegaly in Egypt was a controversial subject and its actual cause remained a puzzle for a long time. The various theories which attribute splenomegaly to *S. mansoni* infection do not explain the occurrence of these forty-eight cases of splenomegaly from Upper Egypt which is a purely *S. haematobium* area.

If the bilharzial ulceration with its associated intestinal toxaemia, or the periportal hepatic cirrhosis with its associated passive congestion in the portal system is the sole cause of endemic splenomegaly it would be difficult to explain the cases coming from Upper Egypt. As a matter of fact, these few cases show the same morphological changes as those from Lower Egypt, moreover, on microscopic examination they were found to contain fertilized *S. haematobium* ova causing characteristic changes, common to all cases of bilharzial splenomegaly, which will be described hereafter.

In the dry districts of Egypt, e.g., the Kassala and Red Sea provinces where dryness prevails all the year round, snails do not exist and bilharzial splenomegaly is unknown, nor is it found in the oases on the western boundaries of Egypt where well-water only is used as the infective snails cannot live in well-water on account of its more or less salty nature.

Owing to local traditions and customs, more prevalent in the rural than in urban districts, women do not usually carry on the more strenuous kinds of agricultural labour involving work in the marshy grounds and are therefore less exposed to infection, although children of both sexes are equally affected.

During the last 30 or 40 years a large amount of work has been done the subject, but for a long time the real cause of the disease was unknown

nd was the subject of controversy, and it was only recently (in 1929) that the pathological changes in the spleen due to bilharzial infection have been described and demonstrated by the writer.

These changes are in the nature of a peculiar form of inflammation characterized by hyperplasia of the red pulp of the spleen generally, and particularly of the mobile as well as of the fixed endothelial elements. In more advanced cases the hyperplasia and the other changes affect the entire reticulo-endothelial system of the spleen. The white pulp, constituting the Malpighian bodies and lymphatic cords, etc., at first shows a certain amount of reaction and hypertrophy which is gradually followed by progressive atrophy as the disease progresses.

The importance of the role played by the spleen in bilharzial infection was not recognized until very recently. This role is connected with the various processes of resistance displayed by the splenic tissues when they become invaded by bilharzia ova. The spleen possesses an enormous number of phago-cytic cells, i.e., mobile endothelium, reticular endothelium and sinus endothelium, also the large germinal mononuclear cells in the centre of the Malpighian bodies.

In other words, with the exception of the small round cells or lymphocytes of the white pulp, the capsule and the trabecular framework, the spleen is a mass of macrophage tissue, and this fact should always be kept in mind when con-sidering bilharzial infections generally.

Most of the cases of splenomegaly hitherto described in the literature deal with the disease in its advanced stage. However, for the proper understanding of the pathological changes it would perhaps be as well to state that three different stages of the disease can be distinguished—the acute, the sub-acute and the advanced. The disease in *the acute or early stage*, is usually associated with other early symptoms due to a recent bilharzial infection such as general malaise, fever and early bladder or intestinal trouble accompanied with the presence in the urine or faeces of bilharzia ova. At this stage the spleen becomes more or less enlarged, reaching from a few finger-breadths below the costal arch to sometimes as far as the level of the umbilicus. This enlargement is due to swelling of the splenic pulp caused by the reaction set up by the fertilized bilharzia ova when they first invade the splenic tissues, and should not be confused with the transient slight enlargement which is a part of the general toxaemia taking place soon after infection with bilharzial cercariae and a long time before the appearance of actual bilharzial symptoms associated with or without the presence of ova in either urine or faeces.

In an ordinary mild and single infection this enlargement will prevail as long as bilharzia ova continue to reach the spleen and it is only when the infection comes to an end, such as that brought about by a timely and adequate treatment, that the spleen returns to its normal dimensions.

It must not be overlooked, however, that cases occur where the first

infiltration of the splenic tissues by bilharzia ova is dealt with by the phagocytic properties of the spleen itself without any outward manifestations that will attract the attention of either the patient or the physician ; in other words, the phagocytic and other defensive capacities of the spleen are sufficient to deal with and eliminate the infective ova and their by-products. A patient so relieved after a first infection would be entirely normal as far as his spleen is concerned, and this is mostly the case with patients of the middle and upper classes who have been only once exposed to infection and this generally an accidental one. But what, unfortunately, takes place in the greater number of cases, i.e., with the poor or "fellaheen" population of the country is that because of their *continuous* exposure to infected water, they are repeatedly infected and, therefore, in most cases suffer from multiple infections at the same time, with the result that the spleen is subjected to a form of continual strain ; and in addition to the normal reactional activities of its macrophages observed in the early or acute stage the reticulum becomes active and hyperplastic, causing a progressive enlargement of the spleen which may reach medially, as far as the umbilicus or beyond it ; and vertically, as far as the crest of the left iliac bone ; this constitutes the *subacute stage* of the disease. When these multiple infections come to an end under appropriate treatment the spleen undoubtedly decreases in size, but it does not return to its normal dimensions as it does when treated successfully in the earlier stages.

The *advanced stage* of the disease is reached when the earlier stages described above continue for a long time, unchecked by treatment or prophylactic measures such as, for example, protection against subsequent infections or even a change of locality. In these advanced cases, the reaction of the reticulum going on for a long time results in a sort of permanent reticulosis. At the same time the other elements forming the framework of the splenic pulp, namely, the capsule and the trabeculae with their contained vessels, also become permanently thickened, especially the small branches of the splenic artery, which are much more affected than the tributaries of the corresponding splenic vein. The elastic tissue and unstriated muscle fibres normally present in the capsule become more or less replaced by ordinary fibrous tissues ; and the spleen does not tend to decrease in size even when, after the normal cessation of an infection or a prolonged course of efficient treatment, the bilharzia ova are no longer present in the urine and faeces. It is this advanced stage which is usually spoken of as " endemic Egyptian splenomegaly." and its cause has hitherto been a subject of controversy as already mentioned.

PATHOLOGY—MACROSCOPIC APPEARANCE.

The spleen, in mild and early cases, weighs from 250 to 300 grammes. In prolonged or chronic cases its average weight is from 1,500 to 2,000 grammes,

and spleens weighing from 3,000 to 5,000 grammes, or even more, are not uncommon.

The spleen enlarges uniformly without losing its peculiar shape or contour, its borders become thickened and rounded, rendering the anterior superior border with its characteristic notch more accentuated and easily detected on palpation. The capsule is of a dark grey colour, and is usually smooth, and stretches uniformly over the enlarged spleen; except when there is an unduly rapid and great enlargement such as is frequently met with in very severe acute or sub-acute cases, when it becomes tense, overstretched and consequently thinner, its colour becoming more reddish on account of the swollen, congested and inflamed red pulp showing through its thinner texture.

In long-standing cases the capsule loses some of its elasticity and becomes permanently thickened, showing signs of local perisplenitis such as ivory white, opaque, smooth and irregular patches, varying in size from that of a split-pea to 3 or 4 cm. in diameter or even more. These are slightly elevated, and of a definite hardness; and sometimes they become cartilagenous. They are generally found on the anterior superior convex aspect of the spleen and in such places as come in contact with the inner surface of the anterior abdominal parietes (Fig. 1, 16).

Rough, irregular adhesions and bands of contractile fibrous tissue are more prevalent in the vicinity of the hilum and the left dome of the diaphragm, binding the enlarged spleen to the adjacent abdominal viscera such as the pancreas, the anterior surface of the left lobe of the liver, the stomach, the peri-nephritic tissue on the left side and other important structures in the neighbourhood such as the omentum, big veins, arteries, nerves, etc. Sometimes these adhesions are so prolific and dense that the spleen is enveloped in a mass of thick irregular adhesions and bound to the abdominal walls as well as to the adjacent viscera (Fig. 1, 32).

Apart from these structural changes in the capsule, various dark, irregular, non-raised areas (causing mottling due to sub-capsular, diffuse or focal haemorrhages) are generally found, especially in early active cases (Fig. 1, 10); also elevated, smooth, dark patches, pointing to recent red or haemorrhagic infarctions, although these latter are very rare.

At the hilum the vessels are enormously enlarged and thickened (especially the splenic artery) and matted together and also attached to other neighbouring structures with well-formed fibrous tissue adhesions.

On section, a characteristic resistant sensation is felt accompanied in very rare instances by a gritty sensation due to the presence of calcareous deposits in old haemorrhages (Fig. 1, 4, 5, 6, 20).

The cut surface is generally on a level with the capsule or slightly raised above it in the early acute stages and during active exacerbations of the disease. It is homogeneous, opaque and dark-red in colour.

In the early active stages the Malpighian bodies becomes hypertrophied

and are easily seen. In the old advanced cases the Malpighian bodies become very much atrophied and are invisible.

The trabeculae and the contained branches of the splenic artery and vein are of a grey white colour and stand out in contrast to the rest of the homogeneous red substance of the spleen. Sometimes scattered, dark, mottled areas are apparent, more particularly in connection with the trabeculae and under the capsule ; these mottlings are due to interstitial haemorrhages occurring in the course of the disease through rupture of the small branches of the splenic artery, as they leave the trabecular sheathing, and of the thin follicular branches of the ellipsoid or central artery ramifications at the periphery of the Malpighian bodies (Fig. 1, *18*).

In bilharzial splenomegaly, unlike many splenic enlargements, thrombosis of the big arteries and veins does not occur. Infarctions when present, are generally simple and of a comparatively small size ; infarctions of larger size are almost unknown.

In cases complicated with chronic malaria, which is also endemic in some bilharzial centres, the colour of the pulp is dark slate due to its impregnation with the melanin pigment of malaria. In these cases the splenic substance also becomes unduly friable.

In cases complicated by general diseases such as typhoid fever, septic conditions and uncompensated heart diseases, etc., the substance of the spleen shows, besides the peculiar appearances of bilharzial inflammation, the characteristic changes peculiar to these general affections, i.e., induration, softening and pigmentation (siderosis) etc.

PATHOLOGY—MICROSCOPIC APPEARANCE.

The body generally is full of wandering phagocytic cells which circulate continually in the tissues and are always ready, on call, to assemble at the point where they are needed.

They differ from the fixed tissue elements in that they refuse to hold together as a solid structure or even to select a particular place for their permanent abode. They are more numerous than elsewhere in the loose sub-cutaneous, submucous and sub-endothelial tissues of membranes such as the pleura, peritoneum, etc., and in the meshes of the fine reticular tissues of the spleen, lymphatic glands and other lymphatic structures. From these tissues they can easily gain access to the blood stream for conveyance to the places or foci where their presence is urgently needed to attenuate, and finally to digest, destroy and neutralize any foreign material of soluble or insoluble nature which might interfere with the general economy of the body.

The spleen, on account of its structure, is very rich in, and supplies the body tissues with, these phagocytic mobile cells which, besides being phagocytic, are
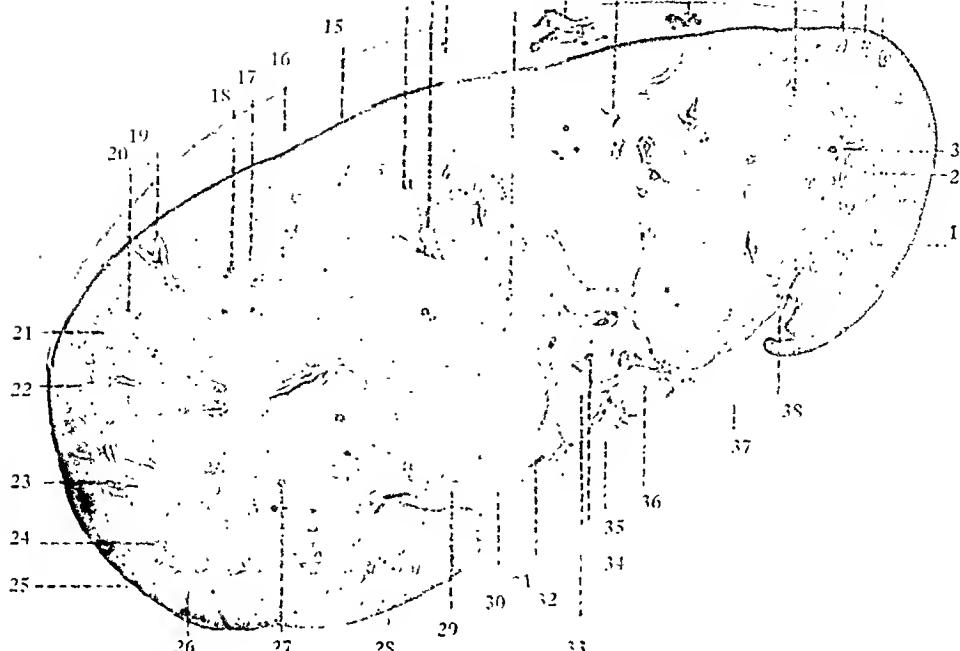
FIG. 1.

FIG. 2.

FIG. 3.

ᵢ. 1.—Sagital section of spleen in sub-acute bilharziasis. × 1/5. Weight, 5 kg., 250 grammes.
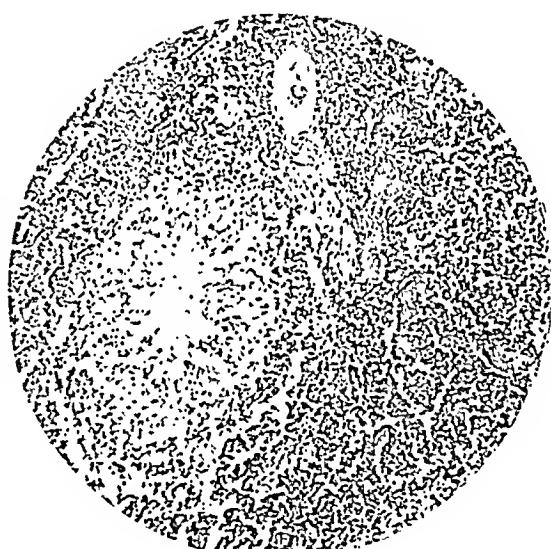
Enlarged Malpighian bodies.	14. Thickened artery.	26. Enlarged Malpighian bodies.
Red pulp.	15. „ capsule.	27. Haemorrhage in Malpighian bodies.
Trabecula with thickened artery.	16. Perisplenitis.	28. Thickened capsule.
Old haemorrhage.	17. Thickened arteries.	29. Splenic vein.
„ „	18. Recent follicular haemorrhages.	30. Adipose tissue.
	19. Trabecula with dilated veins.	31. Thickened splenic artery.
Thickened artery.	20. Haematoidin pigment in old haemorrhage.	32. Capsular adhesions.
Recent subcapsular haemorrhage.		33. Splenic vein.
Thickened artery.	21. Enlarged Malpighian bodies.	34. „ artery.
Recent subcapsular haemorrhage.	22. Haematoidin pigment in old haemorrhage.	35. Adipose tissue.
Splenic vein.		36. Lymphatic gland.
Capsule.	23. Thickened trabecula and vein.	37. Adhesions.
Trabecula with old haemorrhage.	24. Enlarged Malpighian bodies.	38. Splenic vein.
	25. Thickened capsule.	

ᵢ. 2.—Section of spleen. × 100. Fertilized bilharzia ovum penetrating through splenic pulp by its histolytic action immediately before the commencement of endothelial reaction. Notice peculiar empty space around the egg.

ᵢ. 3.—Section of spleen. × 100. Showing a complete ovum (unaffected) and site of a destroyed ovum with the peculiar zone of endothelial proliferation.

FIG. 4.

FIG. 5.

FIG. 4.—Section of spleen in early acute bilharziasis. × 100. 1. Bilharzia ovum with lateral spine (containing embryo). 2. Crushed shell of a destroyed bilharzia ovum. 3. Space between ovum and active endothelial cells. 4 and 5. Sites of digested and absorbed ova surrounded with endothelium. 6. Layers of active endothelial cells. 7. Giant cell. 8. Eosinophilic cells. 9. Splenic sinus.

FIG. 5.—Section of spleen in very early bilharziasis. × 100. 1. Ovum shell. 2. Free embryo. 3. Macrophages. 4. Reticular endothelium. 5. Giant cell. 6. Free embryo (contracted). 7. Trabecula.
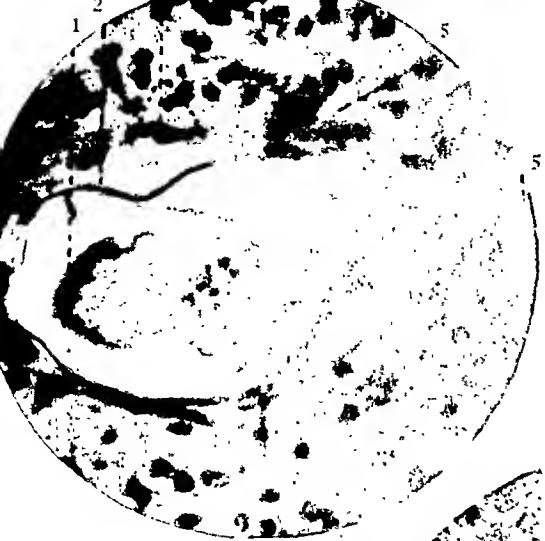
Fig. 6.
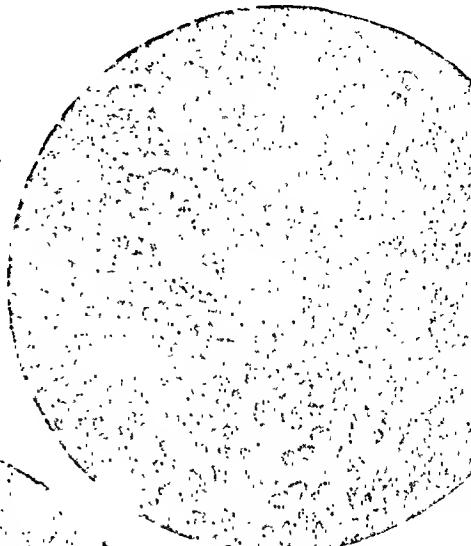
Fig. 7.
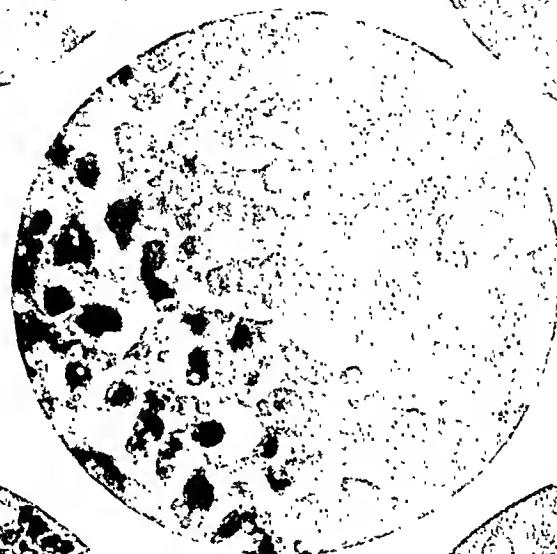
Fig. 8.
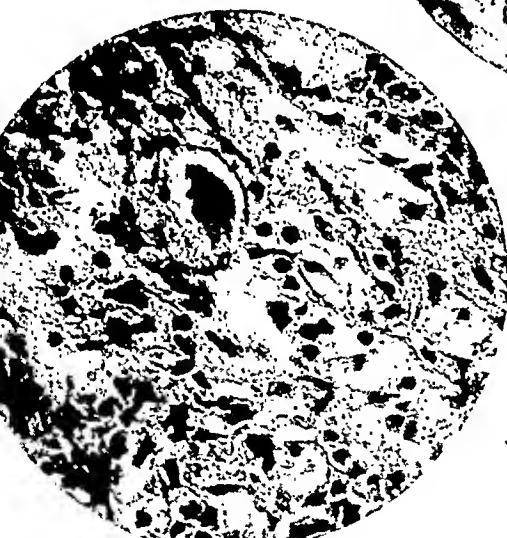
Fig. 9.
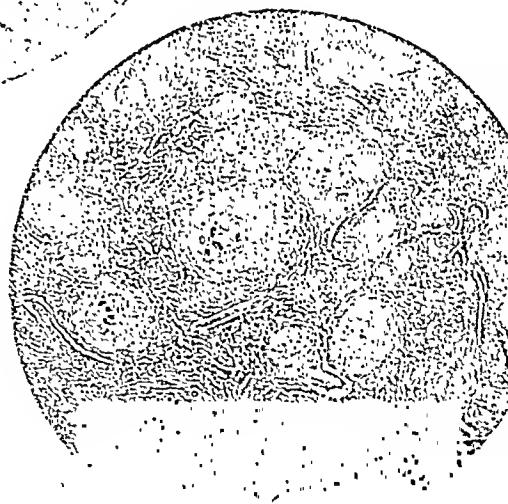
Fig. 10.

Fig. 6.—Section of spleen. × 250. Bilharzia ovum and macrophage reaction. 1. Embryo (miracidium). 2. Shell. 3. Mobile endothelium (macrophages). 4. Eosinophils. 5. Mobile endothelium.

Fig. 7.—Section of spleen. × 100. Partly destroyed ova and characteristic heavy eosinophilic reaction.

Fig. 8.—Section of spleen. × 250. (Active bilharziasis.) Showing heavy eosinophilia almost choking splenic pulp. Note also the peculiar endothelial activity almost filling the sinuses. The small, light vacuoles denote eosinophilic granules.

Fig. 9.—Section of spleen. × 250. Showing characteristic reaction of the sinus and mobile endothelium ; note a large, active mobile cell almost filling a splenic sinus.

Fig. 10.—Section of spleen. × 40. Sub-acute bilharzial spleen showing bilharziomata containing bilharzia ova (dark stained). Note hyperplasia of Malpighian bodies.

FIG. 11.

FIG. 12.

FIG. 13.

FIG. 14A.

FIG. 14B.
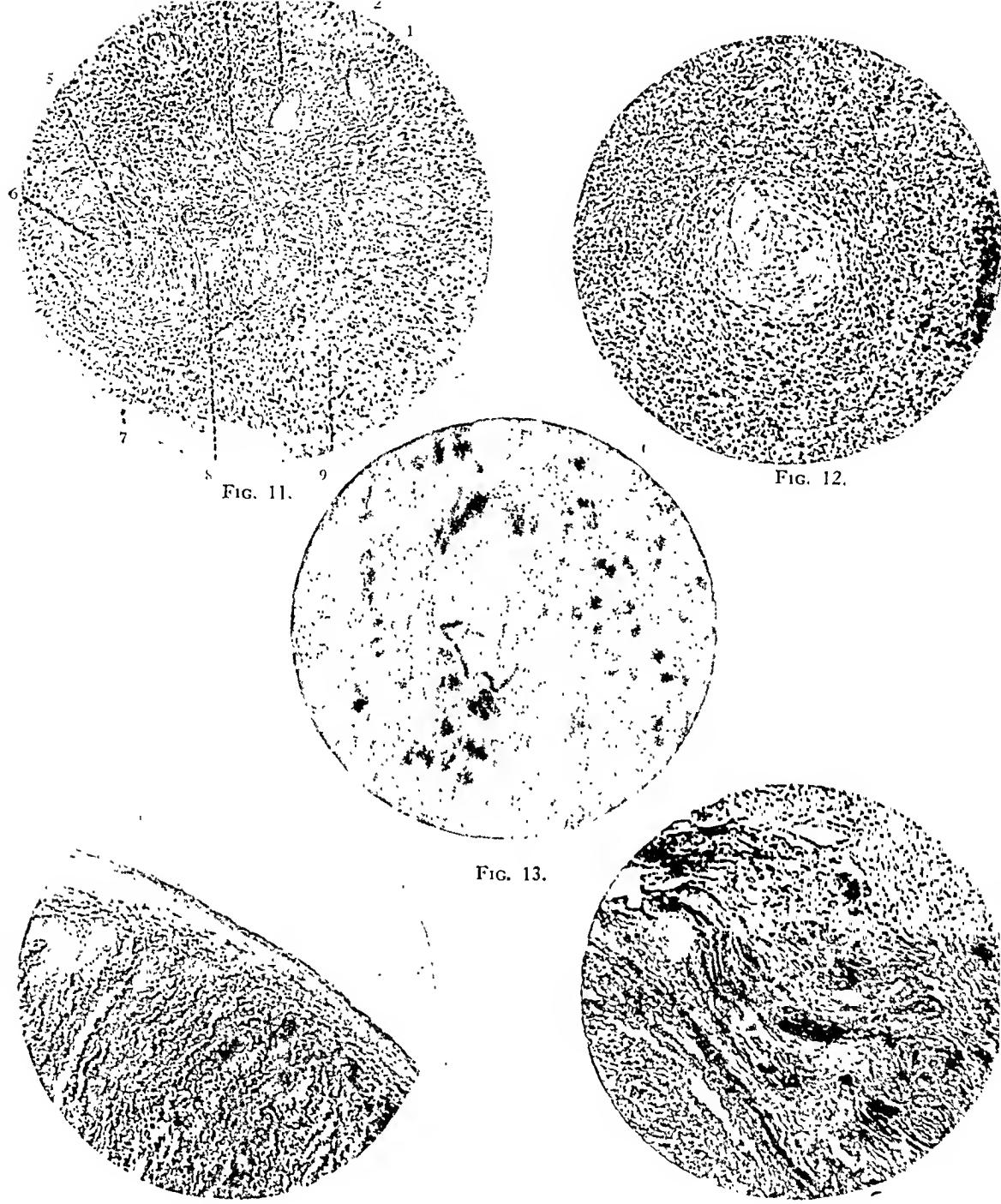
FIG. 11.—Section of spleen in subacute bilharziasis. ✕ 100. 1. Bilharzia ovum with a terminal spine. 2. Giant cell. 3. Broken bilharzia ovum. 4. Hyperplastic endothelium. 5. Remainder of bilharzia ovum. 6. Layers of active endothelium. 7. Splenic sinuses. 8. Giant cells. 9. Eosinophilic cells.

FIG. 12.—Section of spleen. ✕ 100. Showing a giant cell in the act of devouring a *B. haematobium* ovum.

FIG. 13.—Section of spleen. ✕ 250. Showing giant cell containing crushed shell of bilharzia ovum.

FIGS. 14A and 14B.—Section of spleen. ✕ 100. Sub-capsular (A) and focal or trabecular haemorrhage (B). Note activity of reticular cells (dark wavy filaments) pointing towards the centre of the haemorrhage in the attempt at repair.

also highly specialised in producing proteolytic and other liquefying ferments needed for the defence of the body.

What actually takes place when bilharzia ova reach the spleen is that they penetrate through the pulp by their histolytic action and a peculiar form of inflammation rapidly sets in, the varied phenomena and manifold mechanism of which bring into full play with the greatest advantage and in the most opportune concentration such valuable and necessary processes as (*a*) Phagocytosis. (*b*) Chemiotaxis. (*c*) Neutralization of any soluble poisonous byproduct that might be present. (*d*) Later on, such changes and processes as are connected with repair.

Phagocytosis is a rapid accumulation of the phagocytic mobile cells thus reinforcing those already and naturally present in the red pulp. They quickly arrange themselves in concentric layers surrounding the eggs; forming in this way the first line of defence (Figs. 4 and 5).

The egg being in the centre of these macrophages there is a space of variable dimensions, intervening between it and these highly specialised mobile cells. This space most probably contains histolytic elements and other by-products secreted by the bilharzia ova and proteolytic ferments secreted by the surrounding phagocytic cells. The ferments liquefy the egg-shells on the one hand and on the other hand neutralize the poisonous by-product liberated by the bilharzia egg or, more strictly speaking, by the contained embryo (Fig. 6). In this manner these toxic by-products become attentuated before radiating and distributing their offensive properties amongst the cells of the affected part.

The phagocytic cells soon become more active, sending their processes or feelers towards the centre and removing any part of the egg-shell which has more or less resisted the liquefying action of the proteolytic ferments. The latter also neutralize the by-products arising out of the decomposition of the contents of the ova (Fig. 3).

The phagocytic cells then very closely adhere to one another, forming, so to speak, a more or less impermeable barrier hindering the diffusion of any offensive by-product into the healthy cells beyond. They then rapidly multiply and the newly formed cells coalesce, at first in small numbers, forming multinucleated cells with few eccentric nuclei, but later, and at the height of the infective process, they coalesce in larger numbers forming giant cells with many nuclei and a capacious protoplasm big enough to engulf a whole egg (Fig 12) or all that remains of the egg and its contents (Fig. 13). Some of these giant cells are so big as to contain from 70 to 80 nuclei, and even more.

Chemiotaxis.—The soluble products of the invading fertilized ova have a most peculiar power of attracting huge numbers of the polymorphonuclear eosinophilic cells to the seat of infection (" positive chemiotaxis ") and, as in all forms of bilharzial infection, this goes hand in hand with the process of phagocytosis (Figs. 7 and 8).

The by-products of the ova also excite hyperplasia of the bone-marrow and other tissue elements concerned in the making of polymorphonuclear eosinophilic cells ; the latter also then become much increased in the circulating blood.

Extracts of fertilized ova, i.e., embryos, miracidia, etc., when injected experimentally, were found to increase the actual and relative numbers of the eosinophils in the circulating blood and to produce a heavy eosinophilic infiltration into the seat of inoculation. Unfertilized ova were found to be unable to produce such eosinophilia.

When a mass, which is the seat of active bilharzial inflammation and which is duly infiltrated with eosinophilic cells accompanied by a high eosinophilic index in the circulating blood, is removed by surgical intervention, the high eosinophilia in the circulating blood disappears very soon after its removal.

Amongst the other cells also observed in the spleen and especially in the neighbourhood of the invading ova may be mentioned (a) small, round, mononuclear cells or small lymphocytes ; (b) mast cells ; (c) plasma cells ; (d) polymorphonuclear neutrophile cells.

All these cells taken together constitute only a very small minority as compared with the intense eosinophilic cell infiltration taking place in active bilharziasis as already referred to.

Microscopically, there is an obvious general active congestion. The central artery and its branches are dilated and engorged with blood. The fine branches soon become ruptured and cause interstitial haemorrhages, especially in the periphery of the Malpighian bodies ; the latter are swollen and congested.

The mobile, fixed and reticulo-endothelial cells become swollen and show signs of activity or hyperplasia. The large mobile mononucleated phagocytic cells become more numerous and are observed scattered in the splenic sinuses and in the reticular network of the pulp (Figs. 8 and 9).

Other white cells, i.e., small lymphocytes, are also present and very few polymorphonuclear neutrophils are found, especially in the region between the red and white pulp or at the extreme periphery of the Malpighian bodies or that of any still existing white pulp.

Both the fixed and wandering phagocytes in the red pulp may contain a brownish-yellow granular pigment derived from the haemoglobin of the red blood corpuscles which, together with other fixed or mobile cells of the part, have succumbed and were destroyed during the response to the early effects of the infecting ova.

This pigment is easily distinguished by its light brown colour as opposed to the almost black colour of malarial melanin. Again the distribution of melanin is general, i.e., it is present in both the white and the red pulp, whereas the blood pigment prevails more in the red pulp and particularly near haemorrhages. This pigment being a derivative of haemoglobin, contains iron (haemosiderin) and can be easily demonstrated by special staining methods, such as potassium ferro-cyanide and dilute hydrochloric acid, when it takes the blue colour of

sulphate of iron, while malarial pigment does not stain in the same way but remains black.

Focal haemorrhages are also observed at the margin of the Malpighian bodies, in relation with the trabeculae and under the capsule. These haemorrhages are generally of a small size but they sometimes attain considerable dimensions, infiltrating the trabecular tissue to a great extent and a whole Malpighian body may sometimes be replaced by blood (Fig. 14, a and b).

These haemorrhages, on account of their peculiar situation, are due to rupture of either the terminal splenic artery or of the small splenic vein radicles at the point where they leave or enter the trabecular tissue, in other words, when these vessels lose their trabecular support. At this point, these small vessels are so thin and badly supported that they very easily rupture when unduly distended—as during the early period of congestion—because their walls consist only of a thin layer of adventitious tissue (which is very poor in elastic fibres and devoid of muscles) and are only coated with a very thin layer of white pulp or lymphocytes.

These small haemorrhages generally become absorbed and are replaced by newly-formed reticular tissue, in time leaving pigmented patches in the trabecular or capsular tissue ; but very often these haemorrhages are large ones, which, as times goes on, disintegrate and often become calcareous through deposition of calcium salts. At the periphery of the haemorrhages signs of repair soon appear ; the reticular cells become very active and divide and sub-divide, sending long chromatin processes or filaments in every direction in a rosette fashion towards the centres of the haemorrhages. Big endothelial giant cells laden with blood pigment are also prevalent. In very rare instances when the clot is disintegrated and absorbed without organization, its place is marked by a cystic cavity containing clear serous fluid. The walls of the cavity are fibrous and their inner surface is usually stained yellowish brown with haematoidin pigment and contains haemosiderin crystals. The diameter of these cavities is sometimes a few centimetres in length. For a long time the nature of these cavities was unknown.

Associated with the changes just described is the process of repair which is a relatively late event in the bilharzial infection. Repair commences at the margin of the affected focus, just beyond the layers of closely adherent large mononucleated phagocytic cells and giant cell systems, in other words, in the region where the toxic by-products of both the infective agent and the deteriora-ing bodies of the already destroyed cells are so much attenuated that they no longer injure or interfere with the living fixed cells.

On the contrary, these attenuated by-products stimulate the reticular cells which then show signs of activity and proliferation. The fixed cells become generally enlarged, their nuclei become more darkly stained and show karo-kinesis and other signs of cell division.

The newly formed cells are more slender, their processes are filamentous

and branching, as already stated, their nuclei are more elongated, their protoplasm more scanty and morphologically they resemble ordinary fibroblastic cells. These cells are very sensitive and proliferate in their turn if the stimulus is maintained. Their progenies are still more slender with more linear nuclei, less protoplasm and with more branching processes, resembling still more the ordinary newly formed fibrous tissue cells and they are far more sensitive than the mother cells.

When the stimulus ceases to act the process, as a rule, comes to an end, and the cells cease to multiply. The red pulp is now indurated and becomes more dense and is much increased in size.

The fixed cells of the reticulo-endothelial system having enormously increased in number, they become more matted together and the individual cells become far smaller than normal. At the same time the mobile cells become considerably less numerous than normal.

The red pulp, responding to the repeated stimuli of the numerous bilharzial infections, becomes very sensitive and very easily excited, the reticulo-endothelial cells proliferating on the slightest degree of stimulation or even after the stimulating effect of the infecting agent has ceased to act. This explains the continued hyperplasia and progressive enlargement of the spleen observed in cases free from ova or evidence of their presence in any part of the body.

On very rare occasions the normal equilibrium between the different elements of the red pulp is interfered with, resulting in tumour formations. Three of these cases examined during the last five years showed typical endotheliomata. The histological picture is that of bilharzial splenomegaly, except that the endothelial cells of the red pulp had outpaced in activity and regularity of division their intimate collaborators, i.e., the reticular cells of the red pulp, producing a typical picture of endothelioma. In these three cases the history was the same ; they were ordinary cases of bilharzial splenomegaly, who started complaining of more discomfort and sometimes severe pain with an obvious and progressive enlargement of the spleen.

Besides the initial reaction seen in the white pulp, Malpighian bodies, etc., in the early period of the infection, nothing of note is observed except that, as the red pulp proliferates and increases in size, the white pulp atrophies, becoming more scarce and deficient; so much so, in fact, that in advanced cases all that remains of the Malpighian bodies is a thin layer of lymphocytes round the thickened central arteries. The lymphatic processes or cords normally branching and interlacing in the red tissue of the spleen become atrophied and disappear at a comparatively early period.

The trabeculae and the capsule take part in the general enlargement and become uniformly thickened, and in addition to signs of old haemorrhages, such as blood pigmentation (haematin and haemosiderin) calcium and cholesterin deposits, etc., blood effusions of various sizes infiltrating the trabecular and subcapsular tissues are usually observed.

These effusions are due to recent haemorrhages which, unlike those met with during the early stages, are due to the increasing hardness and induration of the red pulp causing obstruction to the open circulation and leading to increased back pressure with distention and rupture of the abnormally thin and badly supported follicular arterioles.

Blood Changes.—The blood changes met with are a progressive secondary anaemia and a distinct leucopenia.

During the early period of the disease there is very little change in the blood picture so far as the red blood corpuscles are concerned, but later on the red blood corpuscles become less in number and continue to decrease with the progressive enlargement of the spleen, until, in advanced cases, the anaemia becomes distinct and sometimes even severe.

The average number of red blood corpuscles in ordinary cases fluctuates between three and four millions, but in some cases when the degree of anaemia becomes extreme, they may only number about 1,500,000 per c.mm. or even less.

When the anaemia is slight there are no perceptible changes in the morphology, size and staining properties of the red cells, but in advanced anaemia changes such as polychromatophilia and megalocythaemia are observed ; nucleated red blood corpuscles are not usually found and no matter how severe the anaemia is, the red blood corpuscles always keep their discoid form.

Unlike those in pernicious anaemia or other severe forms of secondary anaemia, such as are usually met with in ankylostomiasis, the red blood corpuscles in bilharzial splenomegaly do not show degenerative forms such as poikilocytosis and microcytosis, or nuclated forms (normoblasts and megaloblasts). The percentage of haemoglobin is usually very low and the colour index is always below 1.

The white cells are variable during the early active stages of the disease. They show a distinct eosinophilia and the total number of the white cells may become slightly increased, but when the disease is well established, there is a definite leucopenia with a peculiar differential distribution of the various leucocytes.

The polymorphoneutrophil leucocytes become relatively and actually decreased in number, while the non-granular mononuclear leucocytes, although they show a relative increase in total number are really below normal. The polymorphonuclear eosinophils are very variable because, as already pointed out, when the bilharzial infection is active, they increase and when the infection is quiescent they decrease to normal. So also are the large hyaline cells. The average number of white cells in ordinary cases of splenomegaly is from 4,000 to 6,000 leucocytes per c.mm. of blood. The leucopenia becomes more distinct as the disease advances and a count of 2,000 leucocytes per c.mm. of blood is not uncommon in advanced cases. From this one concludes that the leucopenia is really due to the actual decrease of the polymorphonuclear elements and that it goes hand in hand with the progressive enlargement of the spleen, and thus

the hyperplastic red pulp must be interfering in some way or other with the normal fabrication of both red blood corpuscles and leucocytes in the bone marrow and in other haemopoietic structures.   The hyperplastic red pulp must have an inhibitory or toxic action on the haematopoietic function of the blood-forming organs and tissues, because the blood count of both red and white cells rapidly returns to normal after splenectomy, in other words, after the toxic or inhibitory action of the abnormal red pulp of the spleen has totally ceased. The red blood corpuscle count is 4·5 to 5 millions or more, the haemoglobin percentage rises to 70 per cent. or 80 per cent., and such changes as polychromatophilia and megalocythaemia totally disappear.   The red blood corpuscles resume normal diameters from 7·5 to 8μ, and take the appropriate stain uniformly. The white corpuscles become also increased totally while the eosinophilia disappears and the differential count becomes normal.

The differential classification of the leucocytes depends upon the state of the activity of the disease.   In active cases the usual differential picture is as follows :

> Polymorphonuclear neutrophils : 45 per cent.
> Polymorphonuclear eosinophils : 20 per cent.
> Ordinary lymphocytes : 30 per cent.
> Large hyalines : 5 per cent.

If the case is an early one the total count usually keeps within normal limits or it is even slightly increased.

In advanced quiescent cases the picture is as follows :—

> Polymorphonuclear neutrophils : 35 per cent.
> Polymorphonuclear eosinophils : 5 per cent.
> Ordinary lymphocytes : 60 per cent.
> Large hyalines : 2 per cent.

Here there is a distinct and permanent leucopenia.

In advanced cases with exacerbations or re-infection the leucopenia remains, but the differential count approaches that met with in the early conditions, especially as regards the eosinophils which show a more or less definite increase. In other words, by examining a stained blood film from a case of bilharzial spleen one can easily tell if the bilharzial process is still active.

The blood, on the whole, is paler and more hydraemic than normal. It takes longer to coagulate and the coagulum, when completely formed, easily separates from the serum which is of a normal clear straw colour.   Its chemical composition does not vary from that of normal blood, i.e., such inorganic elements as the salts of soda and calcium and such organic elements as urea and cholesterin remain within the normal limits.

It is interesting to note that in the splenic punctures made during the active stage of the disease the material obtained in the syringe is sanguineous, and on microscopic examination, it shows the following :—

(a) Red blood corpuscles constituting the predominating element.

(b) Many eosinophils.

(c) Numerous large hyaline mononuclear cells or splenocytes.

In old quiescent cases material is comparatively difficult to obtain and when examined under the microscope differs enormously from that described above in that the characteristic eosinophilia is absent and the large hyaline cells, although present, are far less numerous, while the other elements, i.e., red blood corpuscles, etc., remain the same.

Changes in the Bone Marrow.—The bone marrow is invariably affected. The femur marrow is harder in consistency, of a dark red colour, being more or less congested ; the myelogenous elements become rarefied and small punctate haemorrhages are sometimes observed.

The rib marrow is comparatively dry and in severe cases becomes scarce and harder than normal in contrast to the soft or diffluent consistency found in advanced cases of other forms of anaemia. Its colour is abnormally dark. Microscopically, in both femur and rib-marrow, the mononuclear non-granular elements show a relative increase, while the polymorpho-granular elements are distinctly diminished, particularly those of the granular neutrophil variety. The marrow cells are less numerous than normal and their nuclei do not show any sign of cell division. Nucleated red blood corpuscles are present but are never abundant, as in pernicious or other severe anaemias.

DISCUSSION.

Several theories have been put forward in explanation of that enlargement of the spleen which is so common in Egypt as to have deserved the name of " Egyptian splenomegaly." Of these theories, however, none stands on so sure a foundation as the one that attributes the condition to the effect of ova deposited during repeated infection by the bilharzia worms. As a matter of fact, it can hardly be called a theory ; it rests on a strong basis of fact.

It was the failure of most observers to find ova causing active changes in the spleen that led to controversy about its etiology. But this failure is really due to the fact that the spleen rapidly destroys ova deposited in it as noted later.

But even among exponents of the bilharzial theory there are those who hold views with which the present writer—on the basis of his findings in hundreds of cases—cannot agree. Such is the view that the splenic enlargement is secondary to bilharzial changes in the liver, or that it results from toxic absorption from intestinal ulcerations of bilharzial origin.

The following facts point irresistibly to the conclusion that " Egyptian splenomegaly " is of bilharzial origin :—

1. The distribution of the disease is that of the infective snails, *Planorbis* and *Bulinus*. It is commonest in the northern provinces of the Nile Delta where drains and canals are numerous and in which snails abound all the year

round. It is much less common in Upper Egypt where, owing to the basin system of irrigation, snails only flourish during the high Nile. In districts which are dry all the year round, e.g., the Red Sea zone and in the western oases, where the well water is unsuitable for the growth of snails, the disease is unknown.

2. The type of persons affected is just that most exposed to infection, i.e., adult men and children of both sexes. Women are less exposed to bilharzial infection on account of the local customs and traditions, and the incidence of splenomegaly in them is relatively slight.

3. All cases give a history, which may be remote or recent, of bilharzial infection.

4. FAIRLEY's complement fixation test and the cutaneous bilharzia reaction are positive in all cases.

CRITICISM OF PAST THEORIES.

The chief theories are :—

1. That the splenic enlargement is due to passive congestion secondary to periportal bilharzial cirrhosis of the liver.

2. That it is even part of a toxic " spleno-hepatic " syndrome.

3. That it is due to toxic absorption from intestinal bilharzial ulcerations.

1. A rise of splenic venous pressure dependent on portal obstruction from hepatic cirrhosis cannot account for the early and marked splenic enlargement, often totally out of proportion to the degree of hepatic involvement, and what is even more significant, unassociated with any of the other characteristic signs of portal hypertension, i.e., dilated veins, haemorrhoids and ascites. Moreover, in the cases from Upper Egypt routine examination of pieces from the liver removed at splenectomy operations showed at most very slight periportal cellular (mononuclear and eosinophilic) infiltrations ; no cases showed marked cirrhotic changes.

2. The absence of the disease in single and accidental infections is against its being in the nature of a response to toxins secreted by the worms, in which response the spleen participates in conjunction with the liver ; in these cases toxins are presumably being formed all the time, and yet no splenomegaly results. It appears that *repeated* infections and the resulting invasion of the splenic tissues by bilharzia ova are necessary for the development of the disease.

3. Intestinal lesions are absent in the *haematobium* infections of Upper Egypt. None of these cases give a history of dysenteric symptoms, even after careful questioning. Thus the presence of intestinal ulcerations, and, therefore, toxic absorptions from these, is not a necessary factor in the development of the disease.

On the other hand, the work here described demonstrates the presence of ova, or the unmistakable sequelae of their former presence, in a considerable

number of cases. If they have not been found more often it is due to the fact that the spleen, on account of its highly phagocytic properties, rapidly destroys and absorbs ova deposited in it. In no part of the human body are bilharzia ova so rapidly destroyed and absorbed as in the spleen ; whereas in the rectum, for example, intact ova are frequently found in sections with a comparatively small cellular reaction around them, in the spleen any ova seen are almost always shattered and destroyed, with an intense surrounding cellular reaction. Characteristic changes, with actual ova in many cases, have been found by the writer in the enlarged spleens of infants from the ages of 3 to 4 months onwards. At this age the condition is particularly fatal, and death is usually ascribed to the various common infantile diseases, especially in view of the severity of the fever and of the intestinal symptoms.

The stage of deposition of ova and the active response of the spleen to this is characterised clinically by a moderate leucocytosis and a marked eosinophilia, fever and splenic enlargement. In addition, there is frequent oscillation of the total leucocyte count, a rise, mostly eosinophilic, presumably occurring with each fresh deposition of ova and the reaction which ensues. These oscillations range from a few hundred to two thousand or more leucocytes, and are noticeable, if daily total leucocyte counts are made. In the non-active or quiescent, as well as in the advanced stages of the disease, this variation in the total leucocyte count disappears and leucopenia persists and keeps at almost the same level. These observations can be of use in deciding between medical treatment and operation.

Once the stage of reticulosis of the pulp with the accompanying fibrosis of the capsule and trabecular framework, has been reached, specific anti-bilharzial treatment—so effective in reducing the size of the spleen in the earlier stages—becomes of no avail.

SUMMARY.

1. The pathological findings in 1,400 cases of endemic Egyptian spleno-megaly are described.

2. The cause of the disease is infection with ova of one or both of the parasites, *Schistosoma haematobium* and *S. mansoni*.

3. The splenic enlargement is due to response of the reticulo-endothelial tissue of the spleen to deposition of ova in it. As a result there is a cellular response which quickly ends in digestion and removal of the solid remains (parts of the egg-shell) of the ova. With frequent repetition of the process, under the condition of perpetual re-infection in which the patients live, there is produced ultimately a condition of permanent hyperplasia and fibrosis.

4. The splenic enlargement is not dependent on either intestinal or hepatic lesions.

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A CLINICAL STUDY OF DIETS IN THREE GOVERNMENT INSTITUTIONS IN KHARTOUM.

BY

H. RICHARDS, M.B., B.S. (LOND.), D.T.M. & H.
*Public Health Section, Sudan Medical Service.**

The major importance of the science of nutrition is yearly becoming increasingly recognized. In 1936, for the first time, a Section of Nutrition was assembled at the Annual Meeting of the British Medical Association. From a mass of work spread over recent years attempts have followed to lay down standards defining minimum dietetic requirements for individuals living under diverse conditions. Such standards can only be obtained from an average and can take no account of the frequently wide disparity between the minimum dietetic requirements of apparently comparable persons. Furthermore, such standards have mainly been derived from figures available in temperate countries. They are not necessarily suitable as measures of comparison for the dietaries of natives in tropical lands.

Conditions in the Sudan are not comparable with conditions in countries in the temperate zone. The Sudanese people are accustomed to a certain kind of diet. Indiscriminate attempts to enrich that diet may not be entirely without danger. It is notable that the adult Sudanese of easy circumstances and subjected to European contact commonly tends to excessive weight and its concomitant evils.

When attempting to compare the calorie yield and composition of Sudan and European diets it must be borne in mind that the average body weight for surface area of the Sudanese Arab is generally somewhat less than that of his European counterpart. It seems reasonable to assume that, in the tropics, a lesser intake of calories is necessary to maintain body temperature than is required in colder countries, leaving a greater proportion of the intake available for the production of bodily energy. Finally, the hours of expenditure of physical energy are, in the Sudan, frequently less than is customary in colder countries. It follows that a calorie yield which, under European conditions would be considered insufficient, may in the tropics be fully adequate.

The minimum dietetic requirement may be defined as the intake of food factors necessary to maintain the individual in an average state of health ; to

sustain his physical equilibrium; and to provide the energy requisite for carrying on his daily mode of life. To which definition must be added in the case of the unadult : the diet must provide for a satisfactory rate of growth and gain in weight.

It is felt that, in the Sudan, precise knowledge is lacking as to the minimum and optimum food requirements of its people. The object of the work recorded in this paper is to point a method by which the adequacy of Sudanese diets may be studied. The inception of medical endeavour in the Sudan is of relatively very recent date. To a young, and as yet skeleton, medical service biochemical evaluation of the common foodstuffs of the country is not yet available.

METHOD OF INVESTIGATION.

The ultimate and only satisfactory method by which the adequacy of a diet may be assessed is by a clinical test. Such a method requires observation upon the food and condition of a controlled and fixed population during a term of years. A diet upon which such a population is maintained in a normally good state of health, on which its members are enabled to carry on their necessary activities, to maintain their physical equilibrium or to grow and gain weight at a satisfactory rate, is for that population an adequate diet though it may fall short of the biochemical standards advocated as minimum requirements in other countries.

In Khartoum Province are three population groups which appear to be admirably suited for the clinical investigation outlined. The inmates of the Central Prison, the boys in the Reformatory, and the pupils in the Gordon Memorial College represent controlled populations upon which a continuity of clinical observations can be maintained. The output of physical energy by members of these groups is regular and to them a known ration is issued which is prepared and eaten with a minimum of waste.

In each of these communities an approximate estimation of the composition and calorie yield of the daily ration as issued has been made. These communities have been under the medical supervision of the writer for upwards of four years. A record of the bodily weight of individual members over periods of varying length has been kept.

In the estimation of dietetic values there is available no analysis of local foodstuffs, milk excepted, to act as a guide. Somewhat comparable figures have been taken from published results of analyses in other tropical countries. The figures for milk are from analyses of average local samples carried out by the Government Chemist.

In the Sudan the unit of weight by which a ration is measured is the dirhem. For purposes of calculation 1 dirhem is taken as equal to 1/9 of an ounce ; 1 ounce as equalling 30 grammes ; 1 gramme of protein as yielding 4 calories ; 1 gramme of fat, 9 calories ; and 1 gramme of carbohydrate, 4 calories. These

figures are not exactly accurate, but in dealing with data which are only approximate they may be sufficiently near.

THE CENTRAL PRISON.

DIET IN THE CENTRAL PRISON.

The daily ration as issued to each able-bodied, third-class prisoner is :—

Flour	.. ..	228 dirhems (makes 300 dirhems of bread).
Lentils	.. ..	35 ,,
Meat	.. ..	20 ,,
Vegetables ..	..	45 ,,
Onions	.. ..	5 ,,
Native butter	..	5 ,,
Salt ..	.. ..	5 ,,

Two limes are issued thrice weekly. Tea and sugar are given twice a month.

The flour in the ration is wholemeal millet flour, meat is lean mutton or beef, and vegetables are native green leaf plants. They are assumed to yield 5 per cent. of carbohydrate.

Calorie value.—This ration is estimated to yield, as issued, 3,573 calories. The majority of those partaking thereof are engaged in fairly arduous manual labour.

Protein content.—The daily ration contains approximately 112 grammes of protein. Only 14 grammes of the protein are of animal origin.

Fat content.—The fat content of the ration is about 50·5 grammes daily ; 18·5 grammes of fat are of animal origin. Although the calorie yield of animal and vegetable fats is similar the former have generally greater value as a food owing to their higher protective qualities.

Carbohydrate content.—The carbohydrate element, the bulk of the ration, is estimated as 667·5 grammes.

Percentage yield of calories.—12·6 per cent. of the total calories yielded by this ration is derived from protein, 12·7 per cent. from fat and 74·7 per cent. from carbohydrate.

Vitamin content.—No precise work is available whereby the vitamin content of Sudan foodstuffs may be assessed. From usual standards it may appear that this diet is low in foods containing Vitamins A and D, whilst there appears to be a sufficiency of Vitamin B complex and, with the issue of limes, Vitamin C.

HEALTH IN THE CENTRAL PRISON.

The standard of health of inmates in the Central Prison compares favourably with that of natives elsewhere in the province and it is certainly maintained at an average level. The able-bodied amongst the prisoners are regularly employed in manual work which is at least as arduous as that undertaken by any other section of the community. During the past 4½ years there has been no outbreak

of epidemic disease amongst the prisoners. In the same period there have been two moderate epidemics of cerebrospinal meningitis and several of respiratory disease throughout the province at large. In-patients in the prison hospital average only two or three weekly, although in a mixed community of this size there is naturally a proportion of chronically infirm. Attendances for out-patient treatment are large, but attention is mainly sought for minor injuries. Severe septic conditions are very rare. No case of disease frankly attributable to dietary deficiency has been recognized during the past $4\frac{1}{2}$ years, though prior to the issue of limes cases of scurvy amongst the prisoners were not uncommon.

Teeth.

The dental condition of more than 300 able-bodied prisoners was investigated : 73 per cent. had a complete set of sound teeth ; 17 per cent. had some caries or apparent gum disease ; 10 per cent. had lost one or more teeth whilst those remaining to them were sound.

Weight.

323 prisoners were weighed on admission and again at periods varying from 2 months to 7 years.

Group 1.—28 men who had spent 2 months in the prison.
    3 showed no change in weight.
    17 showed an average gain of 4·94 lbs.
    8 showed an average loss of 3·5 lbs.

Group 2.—22 men who had been between 2 and 8 months in the prison.
    5 showed no change in weight.
    5 showed an average gain of 4·25 lbs.
    12 showed an average loss of 4·42 lbs.

Group 3.—63 men who entered the prison during 1935.
    3 showed no change in weight.
    24 showed an average gain of 5·08 lbs.
    36 showed an average loss of 5·53 lbs.

Group 4.—80 men who entered the prison during 1934.
    3 showed no change in weight.
    21 showed an average gain of 7·52 lbs.
    56 showed an average loss of 7·34 lbs.

Group 5.—48 men who entered the prison during 1933.
    2 showed no change in weight.
    14 showed an average gain of 4·29 lbs.
    32 showed an average loss of 9·75 lbs.

Group 6.—32 men who entered the prison during 1932.
    3 showed no change in weight.
    6 showed an average gain of 17·83 lbs.
    23 showed an average loss of 10·17 lbs.

Group 7.— 7 men who entered the prison during 1931.
   2 had gained 14 lbs. and 1 lb. respectively.
   5 showed an average loss of 7·2 lbs.

Group 8.—43 men who entered the prison during 1930.
   1 showed no change in weight.
   4 showed an average gain of 11 lbs.
   38 showed an average loss of 9·95 lbs.

Summary.— 20 men had neither gained nor lost weight.
   93 men had gained an average of 6·58 lbs.
   210 men had lost an average of 7·91 lbs.

The average loss of weight shows a progressive increase from 3·5 lbs. in those who had been 2 months in the prison to 10·17 lbs. in those who entered in 1932. The 1931 group is small beyond significance. In the 1930 group, the average loss is 9·95 lbs.

With such comparatively small numbers it is difficult to attach any great moment to an apparently progressive loss of weight over a period of 7 years.

Age.

It was not possible to show that age had any bearing upon gain or loss of weight under prison conditions. Seven men were under 20 years of age on admission to the prison, one had remained stationary in weight, three had gained and three had lost weight.

Work.

Of the 323 men included in this investigation, twenty-three, for reasons of age or physical disability were not engaged in strenuous labour. Of this group, three showed no change in weight, four had gained and sixteen had lost weight.

PRISON DIET COMPARED WITH ACCEPTED STANDARDS.

Compared with the standards such as those advocated for temperate countries by the Ministry of Health, the British Medical Association, or the League of Nations, the diet in the Central Prison seems inadequate. The calorie yield of about 3,500 as issued would certainly be held low for manual labourers in temperate climates. According to such standards the ration is ill-balanced. The total protein content approximates to that generally advocated, but in temperate lands it is usually held that about 50 per cent. of the total protein should be of high biological value. The low total fat content of the diet and the preponderance of carbohydrates is a feature of most tropical dietaries. The scant quantity of fats of animal origin might appear to indicate a deficiency of protective properties.

THE REFORMATORY.

DIET IN THE REFORMATORY.

The boys in the Reformatory are issued the same diet as the inmates of the Central Prison. These boys lead an active life with physical training, games, light manual work and scholastic instruction.

HEALTH IN THE REFORMATORY.

The state of health in this institution is good.   The sickness rate is insignificant.   On admission the physique and state of nutrition of many of the boys is often notably below average.   Without exception a striking improvement is manifest after a few months of Reformatory régime.

Teeth.

At the time of this investigation there were ten boys in the Reformatory. Nine had apparently sound teeth.   Eruption was not retarded.   One boy had lost one permanent molar.

Weight.

There was no record of the weight of one boy on admission.   Of the remainder :—

One, aged 13 on admission, had after 1 month, gained 5 lbs.

One, aged 12 on admission, had after 3 months, gained 2 lbs.

Two, aged 12 and 13 on admission, had after 7 months, gained 15 lbs. and 6 lbs. respectively.

One, aged 14 on admission had, after 10 months, gained 32 lbs.

One, aged 12 on admission, had after 14 months, gained 13 lbs.

One, aged 14 on admission, had after 18 months, gained 14 lbs.

One, aged 11 on admission, had after 26 months, gained 29 lbs.

One, aged 10 on admission, had in 5 years, gained 34 lbs.

The rate of gain in weight is somewhat unlevel, but it is difficult to attach to this any great import.   The rate of gain depends so largely upon the condition of the boy on entry ; and many of these juvenile delinquents have, whilst at large, led a distinctly precarious and hand-to-mouth existence.

GORDON MEMORIAL COLLEGE.

DIET IN THE GORDON MEMORIAL COLLEGE.

The ration as issued daily in this institution is :—

Bread	..	..	217 dirhems.	Vegetables	..	50 dirhems.
Meat	..	..	50 ,,	Rice	..	..	4 ,,
Milk	..	..	36 ,,	Onions	..	$7\frac{1}{3}$,,

In addition to the above constant constituents of the ration, the following items are given on alternate days :—

At breakfast.—Egyptian beans 15 dirhems, sesame oil 3 dirhems ; or lentils $12\frac{1}{2}$ dirhems, native butter 2 dirhems.

At lunch and dinner.—Rice 30 dirhems, native butter 9 dirhems ; or lentils $27\frac{1}{2}$ dirhems, native butter 7 dirhems.

Salt and red pepper are issued daily.   No fruit is given.   It is probable that this ration is occasionally supplemented by private purchases on the part of the students.

Calorie value.—With figures taken from the same sources as in the case of the Central Prison and employing the same method of calculation the average calorie yield of this ration is estimated as 3,017 daily.

Protein content.—The average daily protein content of the diet is approximately 115 grammes, of which 39 grammes are of high biological value.

Fat content.—The average fat content is 58·2 grammes, of which 40 grammes are derived from animal sources.

Percentage yield of calories.—15·3 per cent. of the total calories yielded by the ration is derived from protein; 16·5 per cent. from fat; and 68·2 per cent. from carbohydrate.

Vitamin content.—This ration appears richer in sources of Vitamins A and D than that of the Prison. It might seem that much of its Vitamin C content may be destroyed in the cooking process.

HEALTH OF THE GORDON MEMORIAL COLLEGE.

The writer has carried out five annual medical inspections in the college. The physique, state of nutrition and health of the students during this period have been generally excellent. Excluding a high, but decreasing, incidence of trachoma the number of boys whom it has been necessary to refer for treatment has been small. During this period the college has been free from major epidemic disease. Only one case of pulmonary tuberculosis has been noted.

Teeth.

From 1932 to 1936, the number of boys examined annually in the Gordon Memorial College was 471, 436, 395, 355 and 290 in successive years. During the same period the numbers referred for dental treatment were 14, 25, 21, 16 and 19 : an average of 4·4 per cent.

Weight and Growth.

The writer, in 1935, carried out an investigation into the rate of gain of weight and of growth of students in the Gordon Memorial College. Two groups of boys were studied.

The first group was composed of eighty-eight boys who had completed a year in the college. The second group contained sixty-eight boys who had completed two years in the college.

In the first group the ages of the boys on entering the college ranged from 14 to 20 years. Seventy-eight were between 16 and 18 years old on entry. In this group there was an average gain of weight of 12·2 lbs. and an average increase in height of 1·8 inches. One boy showed no gain of weight. Five boys had not increased in height. No boy had lost weight.

In the second group the ages of the boys on entry to the college were from 15 to 20 years. Fifty-five were between 16 and 18 years old on entry. The average gain in weight was 16·1 lbs. and the average increase of height was

2·94 inches.　Two boys showed a loss of weight.　No boy had failed to increas in height.

In 1936 the investigation was continued to include fifty-nine boys who ha completed 3 years in the college.　In this group the change in weight and heigl which had taken place during the third year of college life was determine Most of the boys in this group were included in the second group above showin the rate of growth during the first 2 years in the college.

The results obtained from the third group are shown according to ag The age refers to that at the time of the investigation and not to the age of entr

Age. Years.	Number who had Gained Weight.	Average Gain in lbs.	Number who had Lost Weight.	Average Loss. lbs.	No Change in Weight.	Number who had Increased in Height.	Average Increase in Inches.	No Change in Height.
17	5	8·3	—	—	—	5	1·65	—
18	11	5	4	2·1	1	15	0·97	1
19	10	4·5	5	3·8	1	14	0·7	2
20	9	3·9	5	2	1	15	0·6	—
21–22	3	2·5	3	1·2	1	5	0·4	2
Total	38	4·8	17	2·4	4	54	0·8	5

THE GORDON MEMORIAL COLLEGE DIET COMPARED WITH ACCEPTED STANDARDS.

This ration is more varied and is theoretically somewhat better balance than that issued in the Central Prison.　It still falls below standards that woul be deemed adequate in temperate lands.

The students in the Gordon Memorial College are, both in age and from th mode and activities of their lives, comparable to those of a British public schoo For the latter, a diet yielding only 3,000 calories daily as issued would be con sidered low.　FRIEND (1936), the Medical Officer at a boys' public school i England (Christ's Hospital) writes : " It is generally accepted that an adult ma doing light work requires on an average a total daily intake of 3,400 calorie A boy of from 14 to 18 years of age requires just as much, some boys needin more and some less according to their height, rate of growth and range o activity."

GIFFARD (1936) published the result of an investigation of the diet of public school over 6 days.　He estimated the yield consumed as 3,354 calorie daily.

The content of first class protein in the Gordon Memorial College die reaches the bare minimum generally accepted in temperate climates.　Th content of total fat and of fat of animal origin is only about half that recom mended in usual standards.　Consequently the percentage of calories derive

from the carbohydrate element of the diet is proportionately high. Both the Gordon Memorial College and the Central Prison rations fall far short of McCarrison's (1936) ideal diet known to ensure perfect nutrition and a high grade of physical efficiency and health in human beings.

Adequacy of the Diets under Consideration.

It has been emphasized that the criteria by which the adequacy of a diet may be judged are obtained not from biochemical analysis but from a clinical test. The criteria suggested are the health of the community partaking of the diet, the maintenance of their physical equilibrium or their rate of growth and the proper performance of their necessary activities. It has been endeavoured to apply these criteria to the diets which have been under consideration.

Judging from standards in temperate countries these diets would not appear to furnish much margin of safety. It is possibly open to question whether they can be held to fulfil the average minimum dietetic requirement.

In the three communities which have been studied the average standard of health attains to at least as high a level as that of the population of the province at large. It is difficult to express an opinion as to whether a higher grade of physical efficiency and health can be achieved by augmentation or alteration of the diet. A view worthy of regard on this point can only be given subsequent to a careful clinical experiment.

The dental condition of these communities is, in the main, excellent. It probably compares very favourably with that of any section of the population of England. The eruption of the teeth and their maintenance in a sound condition is intimately associated with the calcium, phosphorus and Vitamin D metabolism. The diets studied appear to be considerably lower in calcium and phosphorus than the average diets in temperate countries. Meat is taken in small amounts. Milk is either entirely absent or is given in almost negligible quantities. Of the milk-products only native butter is used. This is issued liberally in the Gordon Memorial College, but is little beyond a condiment in the Prison and Reformatory.

The diets, particularly that of the Central Prison are apparently low in foodstuffs containing Vitamin D. It appears probable, however, that the Vitamin D content or potency of foodstuffs produced in a country such as the Sudan, with plenteous sunshine, is higher and more constant than that of foodstuffs grown under cloudier skies.

The sole evidence, possibly suggestive that in some instances the respective diets may fall below minimum requirements, is furnished by the investigation of the weight and rate of growth. The weight, however, is the least reliable of the criteria suggested and the one most open to error, both in execution and of deduction. It is probably sound to place more faith in conclusions drawn from clinical inspection of physique and state of nutrition than from the results of occasional weighing.

In the Central Prison, of 323 men, 65 per cent. had lost weight; 6 per cent. had remained stationary; 29 per cent. had gained weight. The ultimate weighing was carried out during a summer in which climatic conditions seemed unusually trying and at that period of the year when the work of many of the prisoners is at its most arduous.

Obesity and excessive weight are not common amongst those classes of Sudanese from which the inmates of the Central Prison are mainly drawn. With this in view it may appear that the loss of weight recorded in a majority of the prisoners furnishes evidence, at least suggestive, that the prison ration may fall slightly below the minimum dietetic requirement of a number of the prisoners.

The number of boys in the Reformatory is too small to allow the formation of conclusions. Their physical and nutritional conditions on admission are frequently poor. There is, during the period of detention, an invariable improvement in physique and the rate of gain in weight seems to be satisfactory.

Amongst the first two groups of students studied in the Gordon Memorial College there is no indication that the ration is anything but adequate. Of 156 students only two had lost weight and one had failed to gain weight. Five did not show any increase in height.

Amongst the group of fifty-nine senior and generally fuller-grown students 28·8 per cent. had lost weight, 6·8 per cent. had remained stationary in weight and 64·4 per cent. had gained weight. Five boys did not show any increase in height. It may be that the evidence to some extent suggests that, for certain of the bigger boys, the ration falls slightly beneath the minimum dietetic requirement.

SUMMARY.

1. The rations issued in the Central Prison, the Reformatory and the Gordon Memorial College have been studied.

2. An effort has been made to determine the effects of these diets upon the health of the communities concerned.

3. The importance of the clinical method of assessing dietetic adequacy is emphasized.

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THE PATHOLOGY OF THE BRAIN IN RHODESIAN TRYPANOSOMIASIS.*

BY

H. G. CALWELL, B.A., M.D., D.T.M. & H.

Medical Officer, Tanganyika Territory.

INTRODUCTION.

Human trypanosomiasis due to *Trypanosoma rhodesiense* has been found in many African countries. The most extensive outbreak has been in the Tanganyika Territory.

Pathological investigations have been confined to Gambian trypanosomiasis with the exception of those of MACKIE (1934 and 1935) who recorded his findings in three human cases of Rhodesian trypanosomiasis.

The Rhodesian and Gambian forms of the disease differ from one another as a general rule. The former is acute and the latter chronic. In the former polyadenitis is much less marked than in the latter and disturbances of the central nervous system are but little in evidence, while trypanosomes are abundant in the blood, cerebrospinal fluid and lymphatic glands (ZSCHUCKE, 1932).

KLEINE (1928) reported that the *T. rhodesiense* cases he observed in the Mwanza outbreak in the Tanganyika Territory were without doubt clinically distinct from the cases of trypanosomiasis he had observed previously, which were caused by *T. gambiense*.

In dealing with human trypanosomiasis it is, therefore, clear that whatever may be the relationship between the trypanosomes concerned the forms of the disease caused by them differ widely from one another, and it is of interest to see if the clinical differences are reflected in the pathological differences.

The first autopsies in sleeping sickness were recorded by CLARK (1840). He noted the cerebrospinal meningitis. Further accounts were given by GUÉRIN (1869), MOTT (1899 to 1910), THOMAS and BREINL (1905), SPIELMEYER (1907), and BRUCE (1908). All these were dealing with cases caused by *T. gambiense*. MACKIE (1934 and 1935) described three cases of Rhodesian infection. MOTT'S work is the foundation of our knowledge of the neuropathology of human trypanosomiasis.

PERUZZI (1928) made many valuable pathologico-anatomical observations on laboratory animals infected with *T. rhodesiense*.

*This paper is part of a thesis which was accepted by the Queen's University of Belfast for the degree of Doctor of Medicine. It is published with the permission of the Hon. the Director of Medical Services of the Tanganyika Territory.

Material and Technique.

Seventeen human brains were examined. All were obtained by the writer at autopsies he made on Africans who had suffered from Rhodesian trypanosomiasis. More than two years were spent in the collection of the material, for although about 2,000 cases were seen and treated the number who actually died in hospital and whose bodies it was possible to examine was small. All the work except the histological part was done under African bush conditions.

The brains were removed entire. Incisions were made into the lateral ventricles and these were kept open with cotton wool. The whole brain was immersed in 10 per cent. formol-saline.

For histological examination pieces were taken from the frontal, parietal and occipital cortex, the sub-cortical white matter, the corpus callosum, basal ganglia, mid-brain, pons, medulla, cerebellum and choroid plexuses. The tissue was embedded in paraffin wax and sections were cut at 10 to 15μ.

The following stains were used: Delafield's haematoxylin and eosin, Loyez stain, Giemsa's eosin-azur, and toluidin blue.

I do not propose to give complete clinical histories of my cases. The symptoms of the disease are already well known. For the most part the only clinical evidence of involvement of the central nervous system was the abnormal condition of the cerebrospinal fluid and I have, therefore, recorded the findings at lumbar puncture frequently.

Many gaps in the records will be noticed and many instances of insufficient treatment. These are explained by the fact that we were dealing with very primitive people whose contact with European medicine and methods is very recent. Often they came too late for cure and often for one reason or another they gave up treatment too soon. The wonder is not that so many failed to be cured but that in the circumstances of primitive bush life so many were cured. This paper deals with the failures.

Syphilis was not prevalent in the area where I was working and I have discounted it as a cause of serious fallacies in my findings. Fairbairn (1933) reviewed the literature on this subject and concluded that neurosyphilis is exceedingly rare in native races in the tropics.

Case 1.

Mhenwa. Female, aged 18 years.

Duration of illness before diagnosis was $1\frac{1}{2}$ months. Diagnosis was made on 16.5.29, and she died on 28.1.32.

Treatment: 1 gramme of germanin weekly for 3 weeks.

Lumbar puncture: 24.9.29. No trypanosomes in fluid, 3 cells per c.mm.

Blood, 17.1.30. Trypanosomes present.

Treatment: 5 grammes of germanin and 10 grammes of tryparsamide at irregular intervals.

Lumbar puncture : 24.6.30. No trypanosomes, 123 cells per c.mm.

She now had twenty injections of tryparsamide each of 2 grammes and at the end of this course her cerebrospinal fluid contained trypanosomes and the cell count was 246 per c.mm.

Treatment with germanin was started on 27.4.31, but was discontinued after 4 grammes had been given, on account of albuminuria. Six more injections of tryparsamide were given and on 10.7.31 her cerebrospinal fluid was free from trypanosomes, the cells were 93 per c.mm., and the protein was 0·06 per cent. A month later the cells were 230 per c.mm., and the protein was 0·05 per cent. She ran away from hospital and was seen no more until she was brought back to die on 28.1.32.

The duration of her illness was 33 months in spite of ineffective treatment. An untreated patient with a similar *T. rhodesiense* infection would not live more than a year.

The usual clinical signs of a central nervous system involvement were absent throughout.

Autopsy.—On opening the cranium and incising the dura mater there was found a great amount of clear fluid. The dura was adherent to the pia-arachnoid on both sides of the vertex. There were some patches of sticky lymph. The basal dura was not adherent, and there was no exudate on the base. Congestion was absent.

Pieces of the cerebrum, cerebellum and medulla oblongata were sectioned. Perivascular infiltrations were prominent in all sections. No demyelinization was observed. No trypanosomes were found in the tissues.

Case 2.

Maria.—Female, aged 50 years.

Diagnosis was made on 31.10.29, and she died on 9.3.32.

Treatment was begun with germanin 1 gramme weekly for 4 weeks. She went away and was next seen on 16.2.31. Lumbar puncture showed trypanosomes in the fluid, 196 cells per c.mm. and protein 0·075 per cent. She then had 16 weekly injections of tryparsamide each of 2 grammes and in August, 1931, her cerebrospinal fluid was free from trypanosomes, the cells were 30 per c.mm. and the protein was 0·035 per cent. In October, 1931, the cells were 17 per c.mm., and in November, 18 per c.mm.

On 2.3.32, she was again brought to hospital. She was then mentally unsound. There were no signs of an organic lesion of the central nervous system. A lumbar puncture was done and the fluid showed no trypanosomes, 44 cells per c.mm., and 0·05 per cent. protein. No further treatment was given and she died a week later.

Autopsy.—I found the brain very congested. Fluid was abundant. The dura mater was adherent to the vertex of the cranial vault and to the

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pia-arachnoid. Between the olfactory nerves and involving the right nerve was a small tumour. On section it appeared to be a glioma.

Sections were made from several places in the cerebral cortex, the cerebellum and the basal ganglia.

Lesions of trypanosomiasis were found equally distributed throughout. Morular cells were present. Demyelinization and trypanosomes were not found.

Case 3.

Nasizya.—Adult female.

Diagnosis was made on 12.3.32, and she died on 19.3.32. The duration of illness before diagnosis was said to have been 1 month.

When first seen she was waterlogged and appeared to have an acute myocarditis. She was given 1 gramme of germanin on the 1st day and another on the 3rd day. No improvement resulted.

Autopsy.—I found the dura mater free from the skull. On incising it there was an outpouring of clear fluid. There were adhesions to the pia-arachnoid in several places mostly on the vertex. There were sticky patches of milky exudate. Congestion was absent. The ventricles were distended with fluid. No trypanosomes were found in the fluid. The base of the brain appeared normal.

Lesions were found in all sections. They were very plentiful. Morular cells were especially marked in the cerebellum. No demyelinization and no trypanosomes were found.

Case 4.

Sita.—Adult male.

Diagnosis was made on 1.4.32, and he died on 3.4.32. He was said to have been ill for 8 days before diagnosis.

Treatment was 1 gramme of germanin on the day of diagnosis. He became unconscious on the 3rd day and died.

Autopsy.—I found copious fluid under the dura mater. There was a great deal of sticky milky exudate between the dura and the pia mater over the vertex only. Congestion was marked.

Sections were made from the frontal and occipital cortex, basal ganglia, inter-cerebral commissure, medulla and cord.

Perivascular infiltrations were seen in all sections examined. Some perivascular demyelinization was observed.

It is noteworthy that in this case which reached a fatal end so quickly after the onset of the disease changes were found in the central nervous system.

Case 5.

Misozi.—Male, aged 40 years.

Diagnosis was made on 12.4.32. Death occurred on 7.5.32. The duration of illness before diagnosis was said to have been 3 months.

He was given germanin 1 gramme on the 1st, 3rd and 8th days, and 2 grammes on the 15th day.

Lumbar puncture on 28.4.32 showed a normal fluid.

On 6.5.32 he had a severe haematemesis from which he died. This was due to cirrhosis of the liver.

Autopsy.—When the dura mater was incised much straw coloured fluid escaped. There was a sticky exudate along the large vessels of the pia mater on the vertex of both cerebral hemispheres. The meninges were adherent together by this exudate.

Meningitis was very marked in this brain.

Case 6.

Kipoloka.—Adult male.

Diagnosis was made on 17.11.28 and he died on 16.5.32. The record of his early treatment was not available.

I saw him for the first time on 14.8.31 and found trypanosomes in his blood and cerebrospinal fluid; the cells in the latter were 496 per c.mm., and the protein was 0·9 per cent. After 1 gramme of germanin and 2 grammes of tryparsamide, trypanosomes were not found in the cerebrospinal fluid, the cells were 30 per c.mm., and the protein was 0·05 per cent. Treatment was continued with germanin alone as the patient complained of dimness of vision. After receiving 6 grammes of germanin he was again subjected to lumbar puncture. There were no trypanosomes in the fluid, the cells were 28 per c.mm., and the protein was 0·05 per cent. (23.10.31).

He was next seen on 4.1.32. His general condition was good. His vision was normal but he was complaining of severe headache. No trypanosomes were found in his blood or cerebrospinal fluid. The cells in the latter were 86 per c.mm.

He was given a course of a new Bayer preparation, No. 4005, an orally administered arsenical, which was undergoing trial. At the end of the course on 6.2.32, the cells in the cerebrospinal fluid had fallen to 41 per c.mm., but the protein had risen to 0·07 per cent. A month later the cells were 241 per c.mm., and the protein was 0·07 per cent. Another course of germanin and tryparsamide combined was given without benefit. He died on 16.5.32.

Autopsy.—I have no notes of the macroscopic appearance of the brain at autopsy. Sections were made from the cerebral cortex, cerebellum, medulla and cord.

Dilated capillaries and perivascular infiltrations were observed in all sections. The latter were numerous in the cervical cord. Areas of perivascular demyelinization were seen in the sub-cortical white matter.

Case 7.

Mhereguzi.—Adult male.

Diagnosis was made on 20.10.31, and he died on 15.10.32.

The first treatment was 5 weekly injections of 1 gramme of germanin.

On 11.12.31 his cerebrospinal fluid was examined. There were no trypanosomes, 203 cells per c.mm., and 0·09 per cent. protein.

He was then given twelve injections of tryparsamide each of 2 grammes. There was no clinical improvement although the cells in the cerebrospinal fluid fell to 5 per c.mm., and the protein to 0·025 per cent. This was in March, 1932.

In July of the same year he was brought to hospital in a very decrepit state and suffering from dementia. He was given six weekly injections of germanin each of ½ gramme. On 7.10.32 his cerebrospinal fluid was found to be normal. He died a week later after a short period of unconsciousness.

Autopsy.—On opening the dura mater there was an outpouring of clear fluid. The dura was adherent to the underlying meninges along the vertex of both cerebral hemispheres by a sticky matted exudate. Congestion was marked. A small adenoma of the hypophysis was present.

Sections were made from all parts of the brain. Meningitis was marked. Perivascular infiltrations were universal but few in number. Morular cells were very numerous in the cerebellum. Demyelinization was very well marked around vessels in the basal nuclei.

Case 8.

Mizibona.—Adult female.

Diagnosis 11.9.32. Death 5.4.33. Treatment was 5 grammes of germanin, 1 gramme weekly. She was discharged on 18.10.32 apparently normal.

On 8.12.32 she returned and her blood was swarming with trypanosomes. A combined course of germanin and tryparsamide was begun but she ran away after the eighth injection having received 4 grammes of germanin and 8 of tryparsamide. She was brought back on 19.3.33 dying of an extensive necrosis of the jaw and ankylostomiasis. Pneumonia intercurred and she died on 5.4.33. Her blood was free from trypanosomes and her cerebrospinal fluid was normal before death.

Autopsy.—The dura mater was found slightly adherent to the underlying tissue along the vertex of the cerebrum. A lymphatic exudate was present. It was less matted than in many other cases of trypanosomiasis.

The brain was poor in lesions of trypanosomiasis. Perivascular infiltrations were most marked in the basal ganglia and cord. The choroid plexus was fibrotic. Perivascular demyelinization was seen in the medulla.

Case 9.

Dubona.—Adult male.

Diagnosis 24.3.32. Death 11.4.33. Treatment was 1 gramme of germanin weekly for 7 weeks. He was then discharged and was not seen again until he was brought to hospital in the late stage of the disease. He could not tell his

name and his speech was unintelligible. He went into a semi-comatose state and died.

Autopsy.—I found no excess of fluid under the dura mater. Lymphatic exudate was marked along the veins of the pia mater on the vertex of the cerebrum. The dura was adherent by this exudate.

The brain was very rich in perivascular infiltrations. The choroid plexuses appeared normal. The cerebellum was less affected than the cerebrum.

Perivascular demyelinization was most evident in the mid-brain. It was not marked in the cortical areas.

Case 10.

Kubagana.—Male, aged 10 years.

Diagnosis on 24.6.33. Death on 27.8.33. Duration of illness before diagnosis was 2 months.

Treatment was 5 grammes of germanin in five weekly injections each of 1 gramme.

He made a good recovery and was not seen again until 10.7.33 when he was brought to hospital suffering from an abdominal condition which was treated surgically. He died of peritonitis and toxaemia.

Autopsy.—I found the dura mater scantily adherent to the pia-arachnoid on the vertex of the right cerebral hemisphere. There were some flakes of milky exudate. Perivascular infiltrations were found throughout the brain. Morular cells were present in the frontal cortex. The choroid plexuses were oedematous. Perivascular demyelinization was found in the parietal cortex and the medulla.

Case 11.

Rusenebera.—Adult male.

Diagnosis on 3.4.33. Death on 14.9.33. Duration of illness before treatment was 7 months. Treatment was two injections of germanin each of 1 gramme weekly. On 17.4.33 lumbar puncture was done and the fluid was found to be normal. For what reason I do not know, he was given no further treatment. On 14.8.33 he returned and his blood was found to be heavily infected with trypanosomes. He was given 1 gramme of germanin, and lumbar puncture on 20.8.33 showed a trypanosome-free fluid with 13 cells per c.mm. and a normal protein content.

The condition of the fluid argued for a new infection against a relapse for one would have found a very different fluid in a patient suffering from trypanosomiasis of many months' duration.

Treatment with germanin and tryparsamide combined was begun, but he died after only three injections each of 2 grammes of tryparsamide.

He showed signs of dementia for about a fortnight before his death which followed a short period of coma.

Autopsy.—On opening the dura mater there was an outpouring of clear fluid.

The dura was adherent to the pia along the vertex of both cerebral hemispheres. There was no visible exudate. Brain sections showed well-marked

perivascular infiltrations. These were most marked in the parietal and frontal cortex, and sub-cortical white matter and were also prominent in the cerebellar peduncles, basal ganglia and cervical cord.

Morular cells were plentiful in the sub-cortical white matter.

Perivascular demyelinization was seen in the frontal cortex, parietal cortex and pons.

The choroid plexus was free from cellular infiltration. It showed amyloid bodies. In this connection it is noted that the patient was 38 years old.

Case 12.

Bigalagu.—Male, aged 10 years.

Diagnosis on 24.6.33. Death on 27.8.33. Duration of illness before diagnosis was 2 months.

Treatment was five weekly 1 gramme injections of germanin. He made an apparently good recovery. His death was due to peritonitis caused by an abscess of the spleen.

Autopsy.—The dura mater was scantily adherent to the pia on the vertex of the right side. There were some flakes of exudate. Perivascular infiltrations were plentiful. Morular cells were seen in the frontal cortex. The choroid plexuses were oedematous. Perivascular demyelinization was found in the parietal cortex and medulla.

Case 13.

Mbonaucha.—Female, aged 40 years.

Diagnosis on 22.9.33. Death on 1.10.33. Duration of illness before diagnosis was 4 months.

When first seen this woman was very lethargic. She could not walk unassisted. She had a coarse tremor of the hands. The function of the cranial nerves was normal. The knee jerks were absent. No other signs of derangement of the central nervous system were present.

Treatment was 1 gramme of germanin and 2 grammes of tryparsamide.

Autopsy.—I found the dura adherent to the pia-arachnoid along both sides of the superior longitudinal sinus and there was a small amount of sticky exudate. There was also slight adhesion of the dura to the pia on the basal aspect of the cerebellum. This was the only brain in the collection where naked eye basal meningitis was present.

Perivascular infiltrations were well marked throughout the brain. There was marked meningitis of the non-purulent type with round cell infiltration of the meninges. The choroid plexuses showed interstitial changes and a round cell and fibrinous exudate.

Case 14.

Ngenze.—Female, aged 35 years.

Diagnosis on 5.10.33. Death on 15.10.33. The duration of illness before death was 3 months.

Treatment was three injections of germanin, 1 gramme on each of 3 days, the 1st, 3rd and 8th after diagnosis. No lumbar puncture was done.

Autopsy.—On incising the dura a large amount of clear fluid escaped. The dura was adherent to the pia-arachnoid along both sides of the superior longitudinal sinus by a milky sticky exudate. The meninges were greatly thickened. Sections of the brain showed a pneumococcal meningitis and advanced lesions of trypanosomiasis. Perivascular infiltrations were most marked in the frontal, parietal and occipital cortex and the sub-cortical white matter and basal ganglia. No perivascular demyelinization was found. The choroid plexuses showed a mononuclear leucocytic infiltration and fibrosis of the papillary processes. Corpora amylacea were present.

Case 15.

Rusiho.—Male, aged 59 years.

Diagnosis on 22.4.33. Death on 27.11.33.

His first course of treatment was given in a bush hospital by an African. He received 8 grammes of germanin followed by 4 of tryparsamide. I first saw him on 26.6.33. He was so weak that he could hardly walk, but except for dimness of vision there were no signs of impairment of the nervous system. Lumbar puncture was done on the same day and the cerebrospinal fluid was found to be free from trypanosomes. The cells were 62 per c.mm. and the protein was 0·05 per cent.

Further treatment was given consisting of 4 grammes of germanin and 32 of tryparsamide. This course ended on 16.11.33 with no improvement in the patient's condition. He died 11 days later.

Autopsy.—The cerebrospinal fluid was found to be increased greatly. The dura was adherent to the pia-arachnoid along the vertex of the cerebrum by a sticky exudate. The pia-arachnoid was thickened and congested. Perivascular infiltrations were seen in the frontal and occipital cortex, corpus callosum, cerebellar peduncles, mid-brain and basal ganglia. They were most numerous in the last. The medulla was comparatively free. Morular cells were seen in it. Perivascular demyelinization was not found.

Case 16.

Mayoya.—Male, aged 30 years.

Diagnosis on 22.4.32. Death on 16.12.32.

After having received two injections of germanin each of 1 gramme, he ran away and was not seen again until 9.11.32, when he was carried to the hospital in a very weak state. He was very emaciated and had marked ascites. I removed 330 oz. of fluid from his abdomen. I did not find any trypanosomes in it. On the next day I did a lumbar puncture. The fluid was free from trypanosomes. The cells were 333 per c.mm., and the protein was 0·038 per cent. Combined germanin and tryparsamide treatment was given without benefit. He died on 16.12.32.

Autopsy.—I found much fluid under the dura mater. The latter was adherent to the pia-arachnoid over the vertex of the cerebrum behind the rolandic fissure. The adhesions on the right side were denser than those on the left. There was matted exudate with flakes of lymph.

The liver and kidneys were atrophied. The former was cirrhotic and the latter were the seat of a marked nephritic change.

On section the brain did not show many or extensive lesions. There was a small round cell meningitis. Perivascular infiltrations were most marked in the frontal cortex. The choroid plexus was normal. No perivascular demyelinization was seen.

Case 17.

Nyzuko.—Male, aged 40 years.

Diagnosis on 11.2.33. Death on 13.2.33.

Treatment was 1 gramme of germanin on the 1st day. On the 2nd day the patient was unconscious with Cheyne-Stokes breathing and on the 3rd day he died. The duration of illness before diagnosis is unknown.

At autopsy I found the brain very congested. There was a sticky lymphatic exudate binding the dura to the pia-arachnoid, most marked over the vertex of the cerebrum on both sides.

Sections showed perivascular infiltrations most marked in the sub-cortical white matter. They were less marked in the cortical areas, the corpus callosum, the cerebellum and the basal ganglia. They were not seen in the medulla, mid-brain or cord. There was a marked round-cell meningitis.

Perivascular demyelinization was seen in the frontal cortex and the sub-cortical white matter. The choroid plexuses showed corpora amylacea but no cellular infiltration.

THE MENINGEAL LESIONS.

MOTT (1906) found a chronic leptomeningitis in sections of the cerebral cortex, the base of the brain, the cerebellum, and the spinal cord from cases of *T. gambiense* infection. He examined twenty-one brains and found that the meningitis was markedly basal in three, pneumococcal in one and purulent in one. TIDY (1934) and BYAM and ARCHIBALD (1922) quote MOTT as the authority for their statement that in trypanosomiasis the meningitis is usually basal. The figures given by me from his work do not support their statement. MARTIN and DARRÉ (1909) examined the brains from four cases of Gambian trypanosomiasis and found that the meningitis in all of them chiefly affected the vertex of the cerebral hemispheres.

I examined seventeen brains and in only one was there macroscopic basal meningitis ; there was one pneumococcal. With the one exception mentioned, the meningitis was always on top and hardly ever extended more than half way down the sides of the cerebral hemispheres. I never saw meningeal adhesions at the base of the brain. Microscopically, however, there was generalized
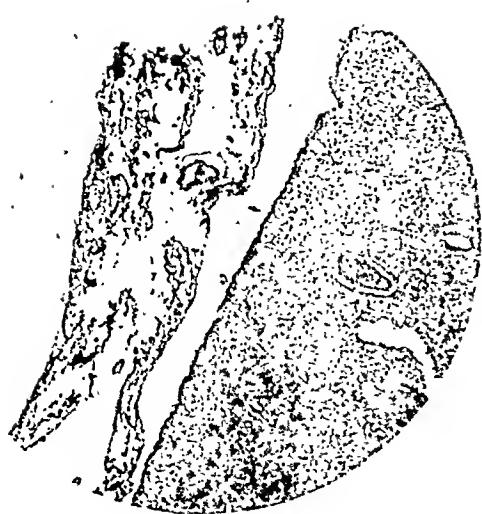
FIG. 1.

FIG. 2.
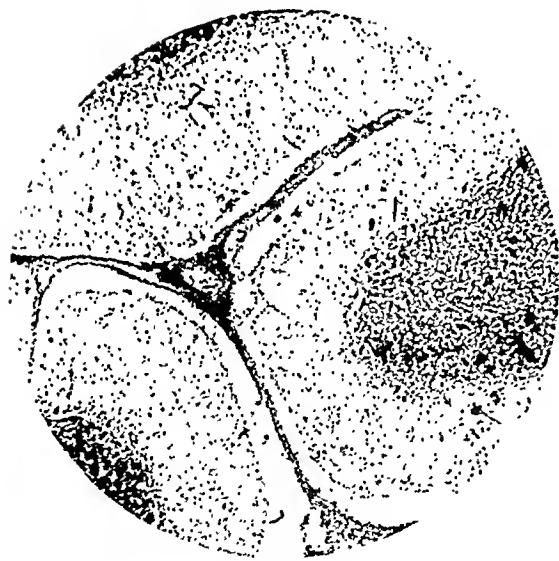
FIG. 3.

FIG. 4.

FIG. 1.—Meningitis.   Infiltration of the pia mater with small, round cells.   × 70.

FIG. 2.—Meningitis.   Dense infiltration of the pia mater with round cells and polymorpho-
nuclear leucocytes.   Dilatation of capillaries.   *Diplococcus pneumoniae* present.   × 70.

FIG. 3.—Meningitis.   Round cell infiltration of the pia-arachnoid which is *seen* dipping
into the sulci of the cerebellum.   × 70.

FIG. 4.—Perivascular infiltration with increase of glial cells in the neighbourhood.   × 106.

FIG. 5.

FIG. 6.

FIG. 7.

FIG. S.
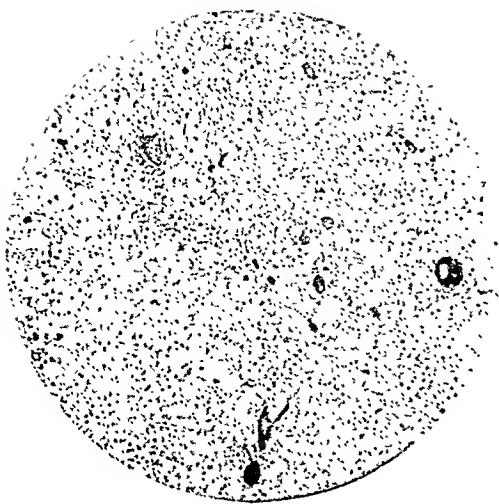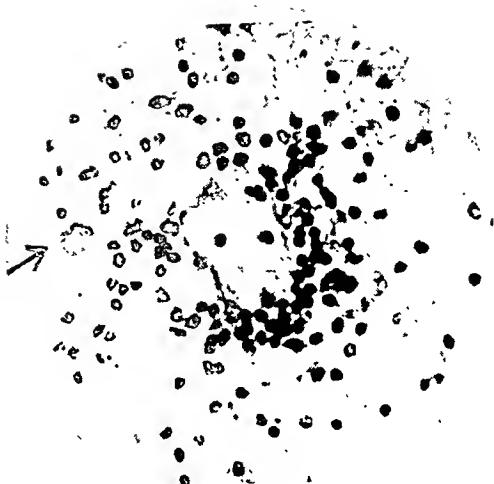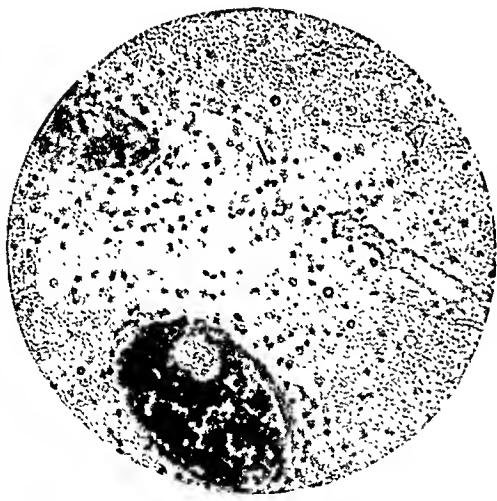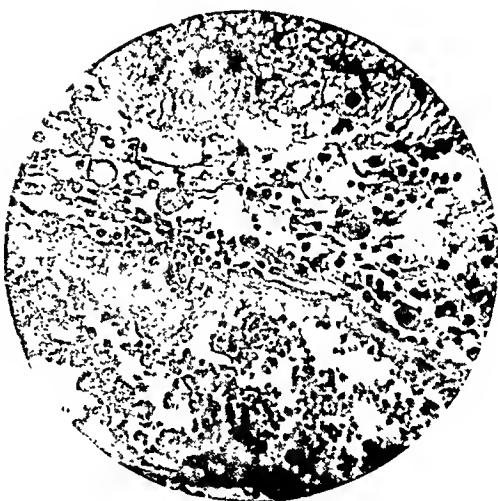
FIG. 5.—Perivascular infiltration. × 70.

FIG. 6.—Perivascular infiltration. Note morular cell. × 450.

FIG. 7.—Perivascular infiltration. Note the smallness of the lumen of the vessel compared with the cell area. × 450.

FIG. S.—Perivascular infiltration. The vessel has been cut obliquely. Red cells are seen in its lumen. Morular cells are seen in the infiltration on both sides of the vessel. At least eight can be seen. A cortical section. × 300.
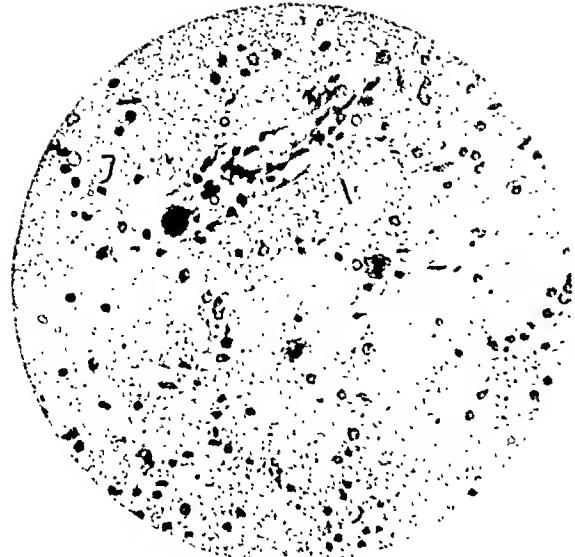
FIG. 9.

FIG. 10.

FIG. 11.

FIG. 12.

FIG. 9.—Morular cells lying free unconnected with a vascular lesion. × 300.
FIG. 10.—Choroid plexus.   Cell infiltration.   × 300.
FIG. 11.—Choroid plexus.   Fibrinous exudate.   × 300.
FIG. 12.—Choroid plexus.   Cell infiltration.   × 300.

PERUZZI (1928) quoted other authorities to show that they were degenerate lymphocytes or derived from the mononuclear cells of the cerebrospinal fluid. In his experimental monkeys infected with *T. rhodesiense* he found the cells in the perivascular infiltrations, in the white matter—frequently near deformed and altered nerve cells—and rarely in the meningeal lesions and on the surface of the pia mater. He concluded that they were analogous to cells found in granulomata and that they have a multiple origin in trypanosomal encephalitis but that the neuroglia is their most frequent origin.

MACKIE (1935) found morular cells most frequent in the cellular exudate of the pia mater. This is in contrast to PERUZZI's statement that they were rare in meningeal lesions (in experimental animals). They were also seen by MACKIE in the perivascular cuffs, and in the brain substance not obviously in connection with blood channels. He has found them outside the nervous system and, therefore, refutes the view that their origin is invariably nerve cells.

I found morular cells in all the brains in my series. They were abundant in eleven and scanty in six. I could not find any relation between abundance or scantiness and other factors.

I found them in every part of the brain except the choroid plexuses. I analysed their distribution in 252 sections and found that they occurred in the following sites in descending order of frequency : medulla, cerebellum, parietal cortex, frontal cortex, occipital cortex, meninges, corpus callosum and basal ganglia.

They were found in the perivascular cuffs and free in the brain substance unconnected with blood channels and as often in the one site as in the other.

I was unable to make out any deformities of the nerve cells in the neighbourhood of morular cells.

THE CHOROID PLEXUSES.

PERUZZI (1928) gave an account of the choroid plexuses in four animals infected with *T. rhodesiense*. He found infiltration with mononuclears, lymphocytes and polymorphonuclear leucocytes, fibrous thickening and deposit of trypanosomes.

FAIRBAIRN (1934) considered that the early changes in the choroid plexuses were responsible for the altered condition of the cerebrospinal fluid in trypanosomiasis. The fluid is also altered by the changes in the vascular endothelium and in the infiltration of the Virchow-Robin space.

I examined the choroid plexuses in eight brains. They were normal in only one brain, and in this one the patient's cerebrospinal fluid was examined a month before death and there were 333 cells per c.mm. The protein was 0·028 per cent. In the others I found round-cell infiltration, fibrosis, fibrinous exudate and oedema.

In Cases 11, 14 and 17, I found many round bodies like corpora amylacea. Their average diameter was 70µ.

Similar bodies were found in the basal ganglia in fatal cases of epidemic encephalitis.

They are commonly found in asylum practice in the brains of old people and especially in the choroid plexuses.

The brains in which I found them were from patients whose age was about 37 years and the average duration of the disease was about 5 months. In two of the brains where corpora amylacea were found other lesions were abundant, and in the third few.

Amyloid bodies are due to degeneration of parenchymatous tissue.

CHANGES IN THE NEURAL ELEMENTS.

There was an increase in glial cells in the neighbourhood of vessels especially those which had infiltrations round them. I did not make any special investigation of the glial cells. MOTT (1906) found no marked pathological changes in them.

I found no changes in the neurones.

I examined sections from fourteen brains for demyelinization and found it in eleven. The areas of demyelinization were all perivascular and were very small but could be found by the low power of the microscope. It was possible to trace the demyelinated fibres right up to the vessel. Such areas were most frequent in the sub-cortical white matter, next in the medulla and pons and least in the cerebellum.

TRYPANOSOMES IN THE TISSUES.

I failed to find trypanosomes in any part of the brain.

MOTT and PERUZZI both found trypanosomes but the former was working when there were no effective trypanocidal drugs and the latter was working with untreated animals whereas all my material was from patients who were under treatment to the end. Even PERUZZI could not always find trypanosomes in the tissues after death although they had been abundant in the living animals immediately before death. Under the most favourable conditions he only obtained good staining of trypanosomes if the material was fixed within 4 or 5 hours after death. MACKIE (1935) failed to find trypanosomes in the tissues of both his *T. rhodesiense* cases. He attributed their absence to the recent administration of tryparsamide.

SUMMARY.

The pathology of Rhodesian trypanosomiasis is studied in seventeen brains of patients who had suffered from the disease.

Case notes are furnished to show the course of the disease as reflected in the condition of the cerebrospinal fluid. The treatment administered is recorded to show how the normally acute Rhodesian form of the disease may be made to

TABLE I.

QUALITATIVE SULPHURIC ACID TEST IN CEREBROSPINAL FLUIDS OF MENTAL PATIENTS.

Case No.	Sex.	Age.	Diagnosis.	No. of Cells.	Globulin Tests.		Total Protein (Nissl)*	Colloid. Gold Test.	Wassermann Test.		H₂SO₄ Test
					Pandy.	Nonne-Appelt.			Cerebro-spinal Fluid.	Blood.	
1	m	29	Mania	2	+	+	3·0	—	—	—	—
2	m	34	Meningitis leutica	90	+++	++	9·0	paretic response	+	+	++
3	f	32	General paralysis	30	+	+	3·0	,,	+	+	+
4	m	45	,,	69	+++	++	8·0	,,	+	+	++
5	m	31	,,	17	++	++	4·0	,,	+	+	+
6	m	51	,,	68	+++	++	5·0	,,	+	+	++
7	f	38	,,	2	+	±	2·0	,,	+	+	+
8	f	38	,,	54	++++	+++	8·0	,,	+	+	++
9	m	45	Tabes	8	++	+	3·5	—	+	+	+
10	f	46	Hysteria	2	—	—	1·0	—	—	—	—
11	f	34	,,	2	—	—	2·0	—	—	—	—
12	m	62	General paralysis	73	+++	++	8·0	paretic response	+	+	+
13	m	43	,,	16	++	+	3·5	,,	+	+	++
14	m	35	,, (?)	5	+	+	2·5	—	not done		—
15	f	27	,,	13	+	++	4·0	paretic response	+	+	+
16	f	48	Melancholia	1	—	—	0·5	—	—	—	—

*This test may be interpreted as follows : Up to 2·0 = normal ; 2·5 = slightly increased ; 3·0 to 4·0 = increased ; above 4·0 = strongly increased.

Further examinations carried out to determine in greater detail the properties of this reaction and its possible practical value gave the following results :—

1. The test can be modified quantitatively by setting up a series of tubes with concentrated sulphuric acid overlaid with various dilutions of cerebrospinal fluid : 1/1, 1/2, 1/4, 1/8, etc. In slightly reacting fluids only the first tube with the undiluted fluid is positive but in many other cases the reaction is given in higher dilutions, the highest positive dilution observed up to now being 1/32. This *degree* of reaction has, as we shall see later, a certain bearing on the prognosis; the test is, therefore, now always carried out in this way up to the dilution 1/64. The *intensity* of colour is not always proportional to the degree of the reaction and has to be taken into consideration as well. We mark a very intense colour as +++, an intense one as ++, a moderate one + and a

weak but still definite one as ±. Vague and indefinite reactions are not recorded. The dilutions have been carried out with normal saline solution; dilutions with distilled water react much more weakly.

2. If the total protein content of the fluid is very high the violet ring gets a brownish hue; that will be marked in the following tables with " b " added to the + signs whilst "b" alone will signify brown colour without violet reaction.

3. The fluid loses its property of giving this reaction within a few days if kept at room temperature, and more slowly if kept in an ice chest. It is, therefore, advisable, especially for the quantitative test, to perform it not later than 24 hours after taking the fluid.

4. Boiling the fluid does not impair the reaction provided that the protein precipitate remains suspended in the fluid. If it is centrifuged the supernatant fluid reacts negatively, the sediment strongly positively with a brownish-violet colour.

5. Shaking a positive fluid with chloroform does not impair the reaction, but extracting it in the same way with ether destroys it completely: the whole mixture divides itself after short standing into three zones, the ether on the top, the extracted fluid on the bottom and a narrow turbid zone between them. Neither the extracted fluid reacts positively nor does the turbid zone, nor the ether extract after evaporation of the ether and redissolution of the residue in normal saline solution, in alcohol, or in the extracted fluid itself.

6. As cholin is frequently increased in pathological fluids it might be supposed that it is this substance that gives the reaction—either alone or in connection with proteins. But the addition of cholin to a positively reacting fluid does not intensify the colour nor does it increase the degree of the reaction; and the test is completely negative with aqueous solutions of various strengths of cholin.

7. Blood serum, as well as egg albumin, gives a weak but similar looking reaction if sufficiently diluted (Table II). It is impossible to say whether these

TABLE II.

SULPHURIC ACID REACTION IN VARIOUS DILUTIONS OF HUMAN SERUM AND EGG ALBUMIN.

Dilutions in Percentages.	100	75	50	25	10	5	4	3	2	1	0·5	0·4	0·3	0·2	0·1
Serum	b	b	b	b	b	—	—	±	±	+	—	—	—	—	—
Albumin	—	—	—	—	—	—	—	—	—	—	+	+	±	±	—

reactions are identical with those in cerebrospinal fluid as the substance causing the reaction is unknown in all three cases. It might be that the reaction is characteristic for certain amine compounds; the fact mentioned already in the

TABLE III.

QUANTITATIVE SULPHURIC ACID TEST IN PATHOLOGICAL CEREBROSPINAL FLUIDS.

Diagnosis.	Case No.	Age in Years.	Sex.	Day of Disease.	Dilutions of Cerebrospinal Fluid.							Final Result.
					1/1	1/2	1/4	1/8	1/16	1/32	1/64	
Epidemic meningitis	1	20	m	5	±	—	—	—	—	—	—	Cured.
	2	½	f	3	±	—	—	—	—	—	—	,,
	3	35	m	3	+	—	—	—	—	—	—	,,
	4	5	f	4	±	±	—	—	—	—	—	,,
	5	½	m	11	+	±	—	—	—	—	—	,,
	6	7	m	7	+	±	—	—	—	—	—	Died.
	7	8	f	7	±	+	—	—	—	—	—	Cured.
	8	4	f	5	±b	±b	—	—	—	—	—	Died.
	9	18	m	2	+·	±	±	—	—	—	—	Cured.
	10	30	m	3	+	±	±	—	—	—	—	,,
	11	5	f	3	+	+	±	—	—	—	—	,,
	12	14	f	2	+	+	±	—	—	—	—	,,
	13	8	m	4	+	+	±	—	—	—	—	,,
	14	6	f	4	+b	±	±	—	—	—	—	Died.
	15	25	m	12	+++	++	+	—	—	—	—	Cured.
	16	13	m	3	+++b	++	±	—	—	—	—	Died.
	17	6	m	3	+	+	+	±	—	—	—	,,
	18	18	m	8	+b	+b	+	±	—	—	—	,,
	19	7	m	2	++	+	+	±	—	—	—	Cured.
	20	2½	f	5	++	++	+	±	—	—	—	Died.
	21	23	f	3	+++b	+b	+	±	—	—	—	,,
	22	20	f	2	+++	++	+	±	—	—	—	,,
	23	20	m	2	+++	++	+	+	—	—	—	Cured.
	24	¼	m	5	++b	+b	+	±	±	—	—	Died.
	25	12	m	3	++	++	++	+	±	—	—	Cured.
	26	40	m	2	+++	+++	++	+	±	—	—	Died.
	27	28	f	8	++	++	++	++	+++	+	—	,,
Poliomyelitis	28	4	m	3	—	—	—	—	—	—	—	Cured.
	29	6	m	4	±	—	—	—	—	—	—	,,
	30	20	m	3	++	+	±	—	—	—	—	,,
	31	20	f	42	+++	++	+	—	—	—	—	Died.
Streptococcal meningitis	32	9	m	10	+b	±b	±b	±	±	—	—	Died.
Typhoid meningitis	33	9	m	3	++b	++	++	++	±	—	—	,,
Pneumococcal meningitis	34	10	m	3	+++	++	+	+	±	—	—	,,

first publication, that it is strongly positive after administration of urotropine, points in the same direction.

8. The results of quantitative tests carried out in twenty-seven cases of epidemic meningitis, four cases of acute poliomyelitis and three cases of meningitis of various origin showed a connection between the degree and intensity of the reaction and lethality. These results are reproduced in Table III and arranged according to the degree of the reaction. It should be noted that the fluid used for it was always the first one taken, before any intrathecal serum treatment was started which might have vitiated the test.

Amongst the first sixteen cases of epidemic meningitis with a positive reaction not higher than a dilution 1 : 4, only four fatalities occurred (25·0 per cent.), whilst out of the other eleven patients showing reactions of higher degrees eight died (72·7 per cent.).

The intensity of colour in the tests with undiluted fluids seems to be of importance only so far as the degree of reaction is usually high in fluids giving an intensely coloured test in the first tube ; but in some cases a weak intensity might go up to rather high dilutions (Cases 17, 18, 32). On the other hand, a strong intensity found in the dilutions seems to be an unfavourable sign.

The appearance of a brownish hue is obviously of very bad significance, as all these cases died.

It seems that this interpretation holds good for poliomyelitis and meningitis of other origin as well, although the number of such cases examined is still too small to allow definite conclusions.

No connection could be found between intensity or degree of the reaction and intensity of meningeal inflammation estimated by the number of leucocytes present in the fluid : some very purulent fluids gave a weak reaction whilst others, but slightly turbid, reacted strongly. The positive tests obtained in general paralysis and poliomyelitis prove that as well ; in these diseases the degenerative changes of the nervous tissue are the main feature rather than the inflammatory ones. Therefore, it seems to us more probable that when the reaction in the sulphuric acid test is positive, this is due to the presence of substances derived from the nervous tissue and that the degree of its destruction might be indicated by the degree and intensity of the reaction.

SOME RESULTS OF TREATMENT OF LEPROSY IN THE SOUTHERN SUDAN.

BY

H. M. WOODMAN, M.B., B.CH.,*

Sudan Medical Service.

The following data apply to the biggest leper settlement in Africa. An analysis is given of some 3,500 cases. A term of 6 years has been allowed to elapse before publication of observations and results, and it is felt that this should be long enough to assess the findings as of some definite value, and, as far as Central African leprosy is concerned, be a definite contribution to the advocacy or otherwise of treatment.

The area in question has been described by CRUICKSHANK (1932).

Country.—The frontiers of the Bahr el Ghazal area of the Sudan and the Belgian Congo, between latitudes 4° and 6° N., open savannah forest, with a rainfall of approximately 51 in., an altitude of 2,000 ft., and a temperature range from 54° to 100° F., covers some 15,000 square miles and carries a population of over 120,000.

Race.—The people are entirely of the mesaticephalic Azande tribe, or its partially absorbed subordinates numbering nearly ten ethnologically related peoples.

F*

Mode of Life.—They are purely agricultural, living on a carbohydrate diet based upon eleusine, manioc, maize, groundnuts, bananas, beans, and forest roots and herbs. They have no meat except in the short hunting season, and are completely deprived of milk.

Historical.—There is no written record of these people earlier than the eighteen forties. The earliest reliable writers hardly mention the existence of leprosy. I cannot recall any reference to it at all by SCHWEINFURTH, YUNKER, CASATI, EMIN and GESSI. It is not, however, regarded as a new endemic disease by the people themselves.

THE CAMPAIGN.

A complete leprosy survey of the area was carried out in 1929-30. Patients were convened into three settlements, the biggest with 2,000 patients. The three main principles were :—

(1) To register and segregate every advanced case, *i.e.*, C2-3 or active N2, and " mixed " advanced cases.

(2) To register and admit all others, from the earliest N1, who were willing to come in.

(3) To register and inspect, but not admit, the remainder and observe them as controls.

The following analysis refers to Li Rangu which is the largest of the settlements.

Up to date, 2,689 lepers have been admitted to the settlement, of whom 204 are segregated.

There are 809 controls outside, not admitted or treated.

Throughout the population there is a leprosy incidence of 2·8 per cent. as an average, and 7 per cent. as a maximum (by chiefs).

Segregation, being carried out rather differently in different parts of the continent, is mentioned in more detail. This particular method, though by no means ideal, has been found, as far as it goes, to meet the conditions. Standard native mud and grass huts are built at 30 metre intervals, standing back 20 metres from an especially isolated road. Each leper has a hut to himself. If an uninfected wife, husband or child elects to remain with the patient, they will live in the adjoining separate hut. Each hut has its own plantation but extra rations are supplied bi-weekly, supplemented by 6 oz. palm oil, simsim, lulu oil or animal fat (when obtainable).

If a leper is so disabled that he cannot help himself at all he has a sub-chief who is responsible for seeing that a next-door neighbour looks after his cultivation. Other relations are not allowed to sojourn, but no attempt is made to prevent them visiting the patient. Two watchmen are employed to check this. The tendency is for an uninfected wife or husband to desert.

On these conditions there is not much difficulty in getting patients to stay.

Treatment.

(1) From the opening of the settlement until 1932 alepol was the standard treatment. Given at first intramuscularly and then intravenously with or without analgesics such as carbolic, courses were continued on standard lines until the weekly injection reached a maximum of 7 c.c. of 4 per cent. solution. These courses were later varied· in combination with sodium hydnocarpate and gynocardate. At the same time macular and nodular areas were treated with 50 per cent. trichloracetic acid.

Those cases which tended to improve reacted more favourably to sodium gynocardate. There was less complaint of pain and less chance of abscess formation. Accordingly :—

(2) 1933 *et seq.*—sodium gynocardate became the standard treatment. The dosage and length of courses varied in certain individual cases and groups of cases but the general procedure adopted was on the following lines :—

Weekly injections of 3 c.c. of 3 per cent. were given for 3 months, followed by 2 weeks rest.

Then 4 c.c. of 3 per cent. for 3 months, followed by 2 weeks rest.

Then 5 c.c. of 3 per cent. for 3 months, followed by 4 weeks rest.

A second similar course of weekly injections of 3 per cent. solution of sodium gynocardate was given, beginning with 4 c.c. and ending with 6 c.c. and with the same intervals of rest.

This course was repeated after the final 4 weeks rest.

Further treatment then commenced with weekly injections of 2 c.c. of 4 per cent. for one month and 3 c.c. for 2 months, followed by 2 weeks rest.

Then 4 c.c. for 3 months and 2 weeks rest up to 5 c.c. of 4 per cent. for 3 months and a final 4 weeks rest.

Cases subsequently continuing on sodium gynocardate injections commence with 3 c.c. of 4 per cent. but do not exceed 5 c.c. of 4 per cent.

Iodine was tried for broken-down nodules. Ulcers were dressed with hydnocarpus oil or iodoform. Hydnestyle employed intradermally on nodular cases with some success was found impracticable through shortage of staff.

(3) 1935 *et seq.* Methylene blue was used for selected segregated cases. Courses are given intravenously commencing with weekly injections of 2 c.c. of 0·5 per cent. solution, working up to 10 c.c. of 2 per cent. solution over a period of 12 months, allowing 2 weeks interval of rest every 2 months. At the end of 18 months a maximum of 5 c.c. of 5 per cent. solution was being given. Such a procedure did not give rise to the toxic symptoms (headache, backache, giddiness or syncope), found if the drug is pushed too liberally.

(4) 1936. Four groups of advanced cases are now being observed and compared while undergoing treatment respectively by (i) trypan blue, (ii) fluorescein and sod. bicarb., (iii) methylene blue in higher concentrations, (iv) sodium gynocardate.

An analysis is given (Table I) of 1,000 cases indicating progress by categories after 3 years of treatment. The eleven categories given have included the most advanced nervous types under " N2." An interesting table (Table II) is given of a series of early untreated cases for comparison.

TABLE I.

ANALYSIS OF OVER 1,000 LEPER CASES UNDER TREATMENT. LI RANGU.

Progress of Patients	Total	C1	C2	C3	N1	N2	C1N1	C1N2	C2N1	C2N2	C3N1	C3N2
Much improved	194	87	29	—	35	6	20	1	10	5	—	1
Improved	268	134	49	1	34	13	22	1	4	15	4	2
Quiescent	325	108	87	1	33	20	28	6	13	14	5	10
Worse	189	18	25	6	11	16	23	10	25	23	11	11
" Cured "	62	36	1	—	21	1	1	—	—	1	—	—
TOTAL	1,038	383	191	8	134	56	94	18	52	58	20	24

TABLE II.

ANALYSIS OF SOME LEPER CASES NOT UNDER TREATMENT.

Progress of Patients	Total	C1	C2	C1N1	N1
Much improved	27	15	11	0	1
Improved	14	6	4	2	1
Quiescent	180	100	34	12	18
Worse	25	15	4	1	4
" Cured "	27	9	5	2	6
TOTAL	273	145	48	17	30

Commentary on Tables I and II.

It will be noted that spontaneous " cures " appear to number about 10 per cent. among the untreated. This is nearly double the percentage (6 per cent.) of " cured " among the treated. But the cures among the untreated have occurred exclusively among the earliest cases, whereas the other group includes all categories. (By " cured " is meant cases in which no visible lesion is any longer apparent, and does not refer to WADE's definition—" arrested without deformity.")

TABLE III.

ANALYSIS OF NON-SEGREGATED CASES UNDER TREATMENT, 3 YEARS LATER.

Progress of Patients	Total	C1	C2	N1	N2	C1N1	C1N2	C2N1	C2N2
" Cured "	1	—	—	1	—	—	—	—	—
Improved	18	2	5	3	1	2	1	2	2
Quiescent	66	12	12	5	8	6	5	9	9
Arrested	15	10	2	—	—	1	—	2	—
Worse	43	1	7	1	8	2	7	6	11
TOTAL	143	25	26	10	17	11	13	19	22

" Arrested " being where the case has remained quiescent over a period of *three years*.

The improved (including both " much improved " and " improved ") constitute 44 per cent. of the treated compared with 15 per cent. of the untreated.

The proportion of those worse among the untreated is twice as great as among the treated. The most disappointing results are with the advanced nodular and " mixed " cases, the majority of whom are worse.

Generally speaking the results weigh clearly in favour of treatment. Conclusions based upon these analyses should be considered in the light of a similar review applied to the subsequent 3 years.

Included in Tables III and IV are those cases contained in Tables I and II, with the exception of those segregated, who are referred to later.

It would be misleading to compare Tables III and IV alone as an index to the efficacy of treatment, for Table III is made up largely of (a) cases who have

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CORRESPONDENCE.

SYMBIONTS IN BLOOD-SUCKING HEMIPTERA.

To the ·Editor, Transactions *of the Royal Society of Tropical Medicine and Hygiene.*

Sir,

In a recent paper, Wigglesworth (1936) refers to the presence of symbionts in certain Triatomidae (*Rhodnius prolixus, Triatoma rubrofasciata* and *T. infestans*) thinking that it was the first time that such microorganisms were discovered in blood-sucking hemipteran bugs.

However, in previous papers (Dias, 1933 and 1934) I have mentioned the occurrence of bacterioids in *T. megista (Panstrongylus megistus)* in the same situation as Wigglesworth found them in *R. prolixus*, i.e., in the cells of the anterior part of the stomach, near the csophagus.

Recently, I encountered those microorganisms in other species, *T. sordida, T. brasiliensis, T. infestans, T. protracta,* and in one species which lives in bird-nests, *Psammolestes coreodes* Bergroth.

In a paper which will appear shortly in the *Memorias do Instituto Oswaldo Cruz,* I shall give further details of my observations.

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Wigglesworth, V. B. (1936). Symbiotic bacteria in a blood-sucking insect, *Rhodnius prolixus* Stal. (Hemiptera, Triatomidae). *Parasitology,* xxviii (2), 284.

I am, etc.,
Emmanuel Dias.

Instituto Oswaldo Cruz,
Rio de Janeiro.
17th February, 1937.

NEPHRITIS IN THERAPEUTIC MALARIA.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

In Dr. MARY E. TYARS's note on "Nephritis in therapeutic malaria," which appeared in the last number of the TRANSACTIONS (Vol. XXX, No. 4, p. 423) I have observed some errors which I wish to rectify.

On page 423, it is stated: "GOLDIE (1930), however, formed the conclusion from his observations on reviewing 8 years' material (nearly 10,000 cases of which 23 per cent. were quartan malaria) that albuminuria can be found in any chronic ease of malaria, but that it is most likely to appear in quartan which is the most chronic and protracted form."

I find no trace of these figures in GOLDIE's paper as given in the references, and I have little doubt that my own observations have been summarized in these words and under GOLDIE's name. Unfortunately if these are supposed to be my figures, we must point out a further and more important error ; I have never stated that 23 per cent. of the close on 10,000 cases of malaria with which my work deals were cases of quartan ! It is, instead, very clearly specified that on a series of 1,247 positive blood films only 2·9 per cent. showed *P. malariae* ; but in 61 cases of nephritis with malaria parasites in their blood, 17, i.e., 28 per cent. were quartan.

These figures, evidently, have quite a different significance.

I am, etc.,

G. GIGLIOLI.

Blairmont, Berbice,
   British Guiana.
19th *February*, 1937.

Dr. GIGLIOLI's letter was forwarded to Dr. MARY TYARS and the reply below has been received.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

DEAR SIR,

It is regretted that, by an error in checking typescript, the insertion of the phrase "from his observations . . . quartan malaria" (which should have been omitted) should have created the impression that the reference to GOLDIE's conclusions might refer to Dr. GIGLIOLI's instead. "GIGLIOLI (1930)" should have appeared in place of "He" at the beginning of the next sentence and also of a subsequent one in place of "GOLDIE." I am glad to have the opportunity of correcting these mistakes.

I am, etc.,

MARY E. TYARS.

St. Bernard's Hospital,
   Southall, Middlesex.
   9th *March*, 1937.

OBITUARY.

THE RT. HON. Sir AUSTEN CHAMBERLAIN, K.G., P.C., M.P. 1863—1937.

By the sudden death of SIR AUSTEN CHAMBERLAIN on 16th March, the Royal Society of Tropical Medicine and Hygiene has lost a distinguished supporter who had been an Honorary Fellow since 1932 when he spoke at the Opening of Manson House by the PRINCE OF WALES.

SIR AUSTEN was at the time of his death Chairman of the Court of Governors and an active member of the Board of Management of the London School of Hygiene and Tropical Medicine.

In this and many other ways he upheld the family tradition, following the example of his father JOSEPH CHAMBERLAIN who, when Colonial Secretary, collaborated with MANSON in the founding of schools of tropical medicine and the development of colonial medical services.

CHALMERS MEDAL 1937 AWARD.

The Council on 18th March considered the nominations received; and the Chalmers Gold Medal was awarded to Professor R. M. GORDON, of the Sir Alfred Jones Research Laboratory, Freetown, Sierra Leone.

The Medal will be presented by the new PRESIDENT at the Annual General Meeting to be held at Manson House on Thursday, 17th June, 1937.

OBITUARY.

THE RT. HON. Sir AUSTEN CHAMBERLAIN, K.G., P.C., M.P. 1863—1937.

By the sudden death of Sir Austen Chamberlain on 16th March, the Royal Society of Tropical Medicine and Hygiene has lost a distinguished supporter who had been an Honorary Fellow since 1932 when he spoke at the Opening of Manson House by the Prince of Wales.

Sir Austen was at the time of his death Chairman of the Court of Governors and an active member of the Board of Management of the London School of Hygiene and Tropical Medicine.

In this and many other ways he upheld the family tradition, following the example of his father Joseph Chamberlain who, when Colonial Secretary, collaborated with Manson in the founding of schools of tropical medicine and the development of colonial medical services.

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